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September 8, 2000

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UTILITY PATENT APPLICATION TRANSMITTAL

Inventor(s): Jonathan P. Duvick, Jacob T. Gilliam, Joyce R. Maddox, Aragula Gururaj Rao, Oswald R. Crasta, Otto Folkerts

Title: Amino Polyol Amine Oxidase Polynucleotides and Related Polypeptides and Methods of Use

APPLICATION ELEMENTS

1. ☐ Fee Transmittal Form (Submit an original, and a duplicate for fee processing)

2. ☒ Specification [Total Pages 83]
   (Preferred arrangement set forth below)
   - Descriptive title of the Invention
   - Cross Reference to Related Applications
   - Background of the Invention
   - Brief Summary of the Invention
   - Brief Description of the Drawings (if filed)
   - Detailed Description
   - Claim(s)
   - Abstract of the Disclosure

3. ☒ Drawing(s) (35 USC 113) [Total Sheets 2]
   a. ☐ Formal
   b. ☒ Informal

4. ☐ Oath or Declaration [Total Pages ____]
   a. ☐ Newly executed (original or copy)
   b. ☐ Copy from a prior application (37 CFR 1.63(d))
   (for continuation/divisional with Box 16 completed)

      i. ☐ DELETION OF INVENTOR(S)
      Signed statement attached deleting inventor(s) named in the prior application, see 37 CFR 1.63(d)(2) and 1.33(b).

5. ☐ Microfiche Computer Program (Appendix)

6. ☒ Nucleotide and/or Amino Acid Sequence Submission (if applicable, all necessary)
   a. ☒ Computer Readable Copy
   b. ☒ Paper Copy (identical to computer copy) [Total Pages 87]
   c. ☒ Statement verifying identity to above copies

ACCOMPANYING APPLICATION PARTS

7. ☐ Assignment Papers (cover sheet & document(s))

8. ☐ 37 CFR 3.73(b) Statement (where there is an assignee) ☐ Power of Attorney

9. ☐ English Translation Document (if applicable)

10. ☒ Information Disclosure Statement (IDS/PTO-1449) ☐ Copies of IDS Citations

11. ☐ Preliminary Amendment

12. ☒ Return Receipt Postcard (MPEP 503) (Should be specifically itemized)

13. ☐ Small Entity Statement(s) ☐ Statement filed in prior application Status still proper and desired

14. ☐ Certified Copy of Priority document(s)

15. ☐ Other:

Attorney Docket No.: 1134R

16. If a **CONTINUING APPLICATION**, check the appropriate box, and supply the requisite information below and in a preliminary amendment:

☐ Continuation ☐ Divisional ☒ Continuation-in-part (CIP) of prior application No. 09/352,159 and 09/352,168.

Prior application information: Examiner _____ Group/Art Unit:

For CONTINUATION or DIVISIONAL APPS only: The entire disclosure of the prior application, from which an oath or declaration is supplied under Box 4b, is considered a part of the disclosure of the accompanying continuation or divisional application and is hereby incorporated by reference. The incorporation can only be relied upon when a portion has been inadvertently omitted from the submitted application parts.

17. **CORRESPONDENCE ADDRESS**

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Respectfully submitted,

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AMINO POLYOL AMINE OXIDASE POLYNUCLEOTIDES AND RELATED POLYPEPTIDES AND METHODS OF USE

Technical Field

The present invention relates generally to the detection and isolation of fumonisin and AP1 degrading enzymes and to compositions and methods for degradation of fumonisin, a structurally related mycotoxin, or its hydrolysis product AP1. This method has broad application in agricultural biotechnology and crop agriculture and in the improvement of food grain quality.

Cross Reference To Related Application

This application is a continuation-in-part of U.S. Application No. 09/352,159, which claims the benefit of U.S. Provisional Application No. 60/135,391, filed May 21, 1999 and U.S. Provisional Application No. 60/092,936, filed July, 15 1998 all of which are hereby incorporated by reference. This application also claims the benefit of U.S. Application No. 09/352,168, which is hereby incorporated by reference.

Background of the Invention

Fungal diseases are common problems in crop agriculture. Many strides have been made against plant diseases as exemplified by the use of hybrid plants, pesticides and improved agricultural practices. However, as any grower or home gardener can attest, the problems of fungal plant disease continue to cause difficulties in plant cultivation. Thus, there is a continuing need for new methods and materials for solving the problems caused by fungal diseases of plants.

These problems can be met through a variety of approaches. For example, the infectious organisms can be controlled through the use of agents that are selectively biocidal for the pathogens. Another method is interference with the mechanism by which the pathogen invades the host crop plant. Yet another method, in the case of pathogens that cause crop losses, is interference with the mechanism by which the pathogen causes

injury to the host crop plant. Still another method, in the case of pathogens that produce toxins that are undesirable to mammals or other animals that feed on the crop plants, is interference with toxin production, storage, or activity. This invention falls into the latter two categories.

Since their discovery and structural elucidation in 1988 (Bezuidenhout *et al.*, Journal *Chem Soc, Chem Commun* 1988: 743-745 (1988)), fumonisins have been recognized as a potentially serious problem in maize-fed livestock. They are linked to several animal toxicoses including leukoencephalomalacia (Marasas, *et al.*, *Onderstepoort Journal of Veterinary Research* 55: 197-204 (1988); Wilson, *et al.*, *American Association of Veterinary Laboratory Diagnosticians: Abstracts 33rd Annual Meeting,* Denver, Colorado, October 7-9, 1990, Madison, Wisconsin, USA) and porcine pulmonary edema (Colvin, *et al.*, *Mycopathologia* 117: 79-82 (1992)). Fumonisins are also suspected carcinogens (Geary W (1971) *Coord Chem Rev* 7: 81; Gelderblom, *et al.*, *Carcinogenesis* 12: 1247-1251 (1991); Gelderblom, *et al.*, *Carcinogenesis* 13: 433-437 (1992)). *Fusarium* isolates in section *Liseola* produce fumonisins in culture at levels from 2 to >4000 ppm (Leslie, *et al.*, *Phytopathology* 82: 341-345 (1992)). Isolates from maize (predominantly mating population A) are among the highest producers of fumonisin. (Leslie *et al.*, *supra*). Fumonisin levels detected in field-grown maize have fluctuated widely depending on location and growing season, but both preharvest and postharvest surveys of field maize have indicated that the potential for high levels of fumonisins exists (Murphy, *et al.*, *J Agr Food Chem* 41: 263-266 (1993)). Surveys of food and feed products have also detected fumonisin (Holcomb, *et al.*, *J Agr Food Chem* 41: 764-767 (1993); Hopmans, *et al.*, *J Agr Food Chem* 41: 1655-1658 (1993); Sydenham, *et al.*, *J Agr Food Chem* 39: 2014-2018 (1991)). The etiology of *Fusarium* ear mold is poorly understood, although physical damage to the ear and certain environmental conditions can contribute to its occurrence (Nelson, *Mycopathologia* 117: 29-36 (1992)). *Fusarium* can be isolated from most field grown maize, even when no visible mold is present. The relationship between seedling infection and stalk and ear diseases caused by *Fusarium* is not clear. Genetic resistance to visible kernel mold has been identified (Gendloff, *et al.*, *Phytopathology* 76: 684-688 (1986); Holley, *et al.*, *Plant Dis* 73: 578-580 (1989)), but the relationship to visible mold to fumonisin production has yet to be elucidated.

Fumonisins have been shown in *in vitro* mammalian cell studies to inhibit sphingolipid biosynthesis through inhibition of the enzyme sphingosine N-acetyl transferase, resulting in the accumulation of the precursor sphinganine (Norred, *et al., Mycopathologia* 117: 73-78 (1992); Wang, *et al., Biol Chem* 266: 14486 (1991); Yoo, *et al., Toxicol Appl Pharmacol* 114: 9-15 (1992); Nelson, *et al., Annu Rev Phytpathol* 31:233-252 (1993)). It is likely that inhibition of this pathway accounts for at least some of fumonisin's toxicity, and support for this comes from measures of sphinganine: sphingosine ratios in animals fed purified fumonisin (Wang, *et al., J Nutr* 122: 1706-1716 (1992)). Fumonisins also affect plant cell growth (Abbas, *et al., Weed Technol* 6: 548-552 (1992); Vanasch, *et al., Phytopathology* 82: 1330-1332 (1992); Vesonder, *et al., Arch Environ Contam Toxicol 23*: 464-467 (1992)). Kuti *et al.*, (Abstract, Annual Meeting American Phytopathological Society, Memphis, TN: APS Press 1993) reported on the ability of exogenously added fumonisins to accelerate disease development and increase sporulation of *Fusarium moniliforme* and *Fusarium oxysporum* on tomato.

Enzymes that degrade the fungal toxin fumonisin to its de-esterified form (e.g. AP1 from FB1) have been identified in US patent no. 5,716,820, issued February 10, 1998, US patent no. 5,792,931, issued August 11, 1998; US patent no. 6,025,188, issued February 15, 2000; and pending US application no. 08/888,950, filed July 7, 1997, and all hereby incorporated by reference. It is understood that AP1 as used here designates the hydrolyzed form of any fumonisin, FB1, FB2, FB3, FB4, or any other AP1-like compounds, including synthetically produced AP1 like compounds, that contain a C-2 or C-1 amine group and one or more adjacent hydroxyl groups. Plants expressing a fumonisin esterase enzyme, infected by fumonisin producing fungus, and tested for fumonisin and AP1 were found to have low levels of fumonisin but high levels of AP1. AP1 is less toxic than fumonisin to plants and probably also to animals but contamination with AP1 is still a concern (Lamprecht, *et al., Phytopathology*, 84:383-391 (1991)). The preferred result would be complete detoxification of fumonisin to a non-toxic form. Therefore enzymes capable of degrading AP1 are necessary for the further detoxification of fumonisin.

The present invention provides newly discovered polynucleotides and related polypeptides of amino polyol amine oxidase (abbreviated APAO, formerly known as AP1 catabolase, US patent no. 5,716,820, *supra,* US patent no. 5,792,931, *supra*; US patent no.

6,025,188, *supra*, pending US application no. 08/888,950, *supra;* trAPAO is the abbreviation for a truncated, but still functional APAO), capable of oxidatively deaminating the AP1 to a compound identified as the 2-oxo derivative of AP1 or its cyclic ketal form (abbreviated as 2-OP, formerly called AP1-N1, US patent no. 5,716,820, US patent no. 5,792,931, US patent no. 6,025,188, *supra*; pending US application no. 08/888,950, *supra*), isolated from *Exophiala spinifera*, ATCC 74269. The partially purified APAO enzyme from *Exophiala spinifera* has little or no activity on intact FB1, a form of fumonisin. However, recombinant APAO enzyme from *Exophiala spinifera*, expressed in *E. coli*, has significant but reduced activity on intact FB1 and other B-series fumonisins. APAO or trAPAO thus could potentially be used without fumonisin esterase since the amine group is the major target for detoxification. Alternatively, fumoninsin esterase and APAO (or trAPAO) can be used together for degrading toxins.

APAO is a type of flavin amine oxidase (EC 1.4.3.4, enzyme class nomeclature, see *Enzyme Nomenclature 1992*, Recommendations of the Nomenclature Committee of the IUBMB on the Nomenclature and Classification of Enzymes, Academic Press, Inc. (1992)). One class of flavin amine oxidases in mammals is known as monoamine oxidases, where they participate in the conversion of amines involved in neuronal function. A prokaryotic flavin amine oxidase that deaminates putrescine has been described (Ishizuka *et al.*, *J. Gen Microbiol.* 139:425-432 (1993)). A single fungal gene, from *Aspergillus niger* has been cloned (Schilling *et al., Mol Gen Genet.* 247:430-438 (1995)). It deaminates a variety of alkyl and aryl amines, but when tested for its ability to oxidize AP1, was found to not contain AP1 oxidizing activity.

The toxicity of fumonisins and their potential widespread occurrence in food and feed makes it imperative to find detoxification or elimination strategies to remove the compound from the food chain.

Summary of the Invention

The present invention provides polynucleotides and related polypeptides of newly discovered APAOs. SEQ ID NO: 5 contains the nucleotide sequence of an active, truncated APAO (trAPAO), SEQ ID NO: 10 contains the nucleotide sequence of trAPAO with an additional lysine and SEQ ID NO: 22, 35, 37, 39, 41, 43, and 45 comprise full length nucleotide sequences of APAOs isolated from different organisms. In addition,

APAO can be modified to eliminate glycosylation sites and/or cysteine residues, for example, see SEQ ID NOS: 32, 48, 50, and 52. Another aspect of the present invention is the method of predicting possible mutagenesis sites on APAO by developing a 3-dimensional model of APAO and then identifying the possible sites that may contribute to misfolding of the protein. The present invention also includes the 3-dimensional model of APAO generated by a computer modeling program, preferably the *Modeler* program. For expression in a plant, the polynucleotide of the present invention can be operably linked to a targeting sequence. It is an object of the present invention to provide transgenic plants comprising the nucleic acids of the present invention.

Therefore, in one aspect, the present invention relates to an isolated APAO encoding polynucleotide ligated to a fumonisin esterase encoding polynucleotide wherein the APAO encoding polynucleotide comprises a member selected from (a) a polynucleotide encoding a polypeptide of the present invention; (b) a polynucleotide having at least 70% sequence identity to the polynucleotides of the present invention; and (c) a polynucleotide of the present invention. The isolated nucleic acid can be DNA. The isolated nucleic acid can also be RNA. Examples of fumonisin esterase genes include, but are not limited to ESP1 and BEST1.

In another aspect, the present invention relates to vectors comprising the polynucleotides of the present invention, including ligated and non-ligated polynucleotides. Also the present invention relates to recombinant expression cassettes, comprising a polynucleotide of the present invention operably linked to a promoter.

In another aspect, the present invention is directed to a host cell into which has been introduced the polynucleotides of the present invention, including a host cell comprising a fumonisin esterase ligated to an APAO or a fumonisin esterase not ligated to an APAO.

In yet another aspect, the present invention relates to a transgenic plant or plant cell comprising a recombinant expression cassette with a promoter operably linked to any of the isolated polynucleotides of the present invention. Preferred plants containing the recombinant expression cassette of the present invention include but are not limited to maize, soybean, sunflower, sorghum, canola, wheat, alfalfa, cotton, rice, barley, tomato, and millet. The present invention also provides transgenic seed from the transgenic plant.

In another aspect, the present invention relates to an isolated protein selected from the group consisting of (a) a polypeptide comprising at least 70% sequence identity to a polypeptide of the present invention; (b) a polypeptide encoded by a nucleic acid of the present invention; and (c) a polypeptide characterized by a polypeptide of the present invention.

This invention further provides methods of degrading fumonisin, a structurally related mycotoxin, a fumonisin breakdown product, or a structurally related mycotoxin breakdown product, by applying APAO as a spray or wash. Additionally, fumonisins and related mycotoxins can be degraded by the application of both fumonisin esterase enzymes and APAO enzymes. Mycotoxins can be degraded in harvested grain, during the processing of harvested grain, in animal feed, or in plant tissue as, for example, during the use of the plant for silage or as a spray on grain, fruit or vegetables. Further, this invention provides methods of degrading fumonisin, a structurally related mycotoxin, a fumonisin breakdown product, or a structurally related mycotoxin breakdown product, by transforming the APAO polynucleotide, alone or in combination with polynucleotides encoding a fumonisin esterase, into plant cells.

The polynucleotides of the present invention can also be used as a selectable marker for plant transformation. By transforming plant cells with an expression cassette comprising a polynucleotide of the present invention and then placing the plant cells on media containing FB1, AP1 or a phytotoxic analog, only the plant cells expressing the polynucleotide of the present invention would survive.

Another embodiment of the present invention is the use of the enzyme fumonisin esterase and APAO by themselves or in combination as reagents for detecting fumonisin and structurally related toxins.

Brief Description of the Drawings

Figure 1 shows a 3-dimensional model of APAO (1B) based on the crystal structure of a related amine oxidase from maize, maize polyamine oxidase (MPAO) (1A). The sites for possible mutation of APAO to alter glycosylation sites or cysteine residues are shown.

Figure 2 shows a 3-dimensional model of APAO (2B) based on the crystal structure of a related amine oxidase from maize MPAO (2B). The substrate binding holes are shown as circles.

Detailed Description of the Invention

Unless defined otherwise, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Unless mentioned otherwise, the techniques employed or contemplated herein are standard methodologies well known to one of ordinary skill in the art. The materials, methods and examples are illustrative only and not limiting. The following is presented by way of illustration and is not intended to limit the scope of the invention.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of botany, microbiology, tissue culture, molecular biology, chemistry, biochemistry and recombinant DNA technology, which are within the skill of the art. Such techniques are explained fully in the literature. See, *e.g.*, J. H. Langenheim and K. V. Thimann, *Botany: Plant Biology and Its Relation to Human Affairs* (1982) John Wiley; *Cell Culture and Somatic Cell Genetics of Plants*, Vol. 1 (I. K. Vasil, ed. 1984); R. V. Stanier, J. L. Ingraham, M. L. Wheelis, and P. R. Painter, *The Microbial World*, (1986) 5th Ed., Prentice-Hall; O. D. Dhringra and J. B. Sinclair, *Basic Plant Pathology Methods*, (1985) CRC Press; Maniatis, Fritsch & Sambrook, *Molecular Cloning: A Laboratory Manual* (1982); *DNA Cloning*, Vols. I and II (D. N. Glover ed. 1985); *Oligonucleotide Synthesis* (M. J. Gait ed. 1984); *Nucleic Acid Hybridization* (B. D. Hames & S. J. Higgins eds. 1984); and the series *Methods in Enzymology* (S. Colowick and N. Kaplan, eds., Academic Press, Inc.).

Units, prefixes, and symbols may be denoted in their SI accepted form. Unless otherwise indicated, nucleic acids are written left to right in 5' to 3' orientation; amino acid sequences are written left to right in amino to carboxy orientation, respectively. Numeric ranges are inclusive of the numbers defining the range. Amino acids may be referred to herein by either their commonly known three letter symbols or by the one-letter symbols recommended by the IUPAC-IUB Biochemical Nomenclature Commission. Nucleotides, likewise, may be referred to by their commonly accepted single-letter codes. The terms defined below are more fully defined by reference to the specification as a whole.

In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

By "microbe" is meant any microorganism (including both eukaryotic and prokaryotic microorganisms), such as fungi, yeast, bacteria, actinomycetes, algae and protozoa, as well as other unicellular structures.

A "fumonisin-producing microbe" is any microbe capable of producing the mycotoxin fumonisin or analogs thereof. Such microbes are generally members of the fungal genus *Fusarium*, as well as recombinantly derived organisms, which have been genetically altered to enable them to produce fumonisin or analogs thereof.

By "degrading fumonisin" is meant any modification to fumonisin, AP1, or any derivative of fumonisin or AP1 which causes a decrease or loss in its toxic activity, such as degradation to less than 1%, 5%, 10%, or 50% of original toxicity, with less than 10% being preferred. Such a change can comprise cleavage of any of the various bonds, oxidation, reduction, the addition or deletion of a chemical moiety, or any other change that affects the activity of the molecule. In a preferred embodiment, the modification includes hydrolysis of the ester linkage in the molecule as a first step and then oxidative deamination. Furthermore, chemically altered fumonisin can be isolated from cultures of microbes that produce an enzyme of this invention, such as growing the organisms on media containing radioactively-labeled fumonisin, tracing the label, and isolating the degraded toxin for further study. The degraded fumonisin can be compared to the active compound for its phytotoxicity or mammalian toxicity in known sensitive species, such as porcines, rabbits, and equines or in cell or tissue culture assays. Such toxicity assays are known in the art. For example, in plants a whole leaf bioassay can be used in which solutions of the active and inactive compound are applied to the leaves of sensitive plants. The leaves may be treated *in situ* or, alternatively, excised leaves may be used. The relative toxicity of the compounds can be estimated by grading the ensuing damage to the plant tissues and by measuring the size of lesions formed within a given time period. Other known assays can be performed at the cellular level, employing standard tissue culture methodologies e.g., using cell suspension cultures.

By “fumonisin esterase” is meant any enzyme capable of hydrolysis of the ester linkage in fumonisin or a structurally similar molecule such as AAL toxin. Two examples of such enzymes are ESP1 and BEST1 found in US patent no. 5,716,820, issued February 10, 1998; US patent no. 5,792,931, issued August 11, 1998; US patent no. 6,025,188, issued February 15, 2000; and pending US application no. 08/888,950, filed July 7, 1997.

By "structurally related mycotoxin" is meant any mycotoxin having a chemical structure related to a fumonisin or AP1 such as AAL toxin, fumonisin B1, fumonisin B2, fumonisin B3, fumonisin B4, fumonisin C1, fumonisin A1 and A2, and their analogs or hydrolyzed forms, as well as other mycotoxins having similar chemical structures, including synthetically made analogs that contain a C-2 or C-1 amine group and one or more adjacent hydroxyl groups, that would be expected to be degraded by the activity of an enzyme of the present invention. The present invention is the first flavin amine oxidase known to attack a primary amine not located at C-1 (i.e. C-2 of AP1) and resulting in a keto rather than an aldehydic product.

It is understood that “AP1” or “amino polyol” as used here is to designate the hydrolyzed form of any fumonisin, FB1, FB2, FB3, FB4, AAL, or any other AP1-like compound, including a compound made synthetically, that contains a C-2 or C-1 amine group and one or more adjacent hydroxyl groups.

By "amplified" is meant the construction of multiple copies of a nucleic acid sequence or multiple copies complementary to the nucleic acid sequence using at least one of the nucleic acid sequences as a template. Amplification systems include the polymerase chain reaction (PCR) system, ligase chain reaction (LCR) system, nucleic acid sequence based amplification (NASBA, Cangene, Mississauga, Ontario), Q-*Beta* Replicase systems, transcription-based amplification system (TAS), and strand displacement amplification (SDA). See, e.g., *Diagnostic Molecular Microbiology: Principles and Applications*, D. H. Persing *et al.*, Ed., American Society for Microbiology, Washington, DC (1993). The product of amplification is termed an amplicon.

The term "conservatively modified variants" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refer to those nucleic acids that encode identical or conservatively modified variants of the amino acid sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are "silent variations" and represent one species of conservatively modified variation. Every nucleic acid sequence herein that encodes a polypeptide also

describes every possible silent variation of the nucleic acid. One of ordinary skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, one exception is *Micrococcus rubens*, for which GTG is the methionine codon (Ishizuka, *et al., J. Gen'l Microbiol*, 139:425-432 (1993)) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid, which encodes a polypeptide of the present invention, is implicit in each described polypeptide sequence and incorporated herein by reference.

As to amino acid sequences, one of skill will recognize that individual substitutions, deletions or additions to a nucleic acid, peptide, polypeptide, or protein sequence which alters, adds or deletes a single amino acid or a small percentage of amino acids in the encoded sequence is a "conservatively modified variant" when the alteration results in the substitution of an amino acid with a chemically similar amino acid. Thus, any number of amino acid residues selected from the group of integers consisting of from 1 to 15 can be so altered. Thus, for example, 1, 2, 3, 4, 5, 7, or 10 alterations can be made. Conservatively modified variants typically provide similar biological activity as the unmodified polypeptide sequence from which they are derived. For example, substrate specificity, enzyme activity, or ligand/receptor binding is generally at least 30%, 40%, 50%, 60%, 70%, 80%, or 90%, preferably 60-90% of the native protein for it's native substrate. Conservative substitution tables providing functionally similar amino acids are well known in the art.

The following six groups each contain amino acids that are conservative substitutions for one another:

1) Alanine (A), Serine (S), Threonine (T);
2) Aspartic acid (D), Glutamic acid (E);
3) Asparagine (N), Glutamine (Q);
4) Arginine (R), Lysine (K);
5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); and
6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W).

See also, Creighton (1984) Proteins W.H. Freeman and Company.

As used herein, "consisting essentially of" means the inclusion of additional sequences to an object polynucleotide where the additional sequences do not selectively hybridize, under stringent hybridization conditions, to the same cDNA as the

polynucleotide and where the hybridization conditions include a wash step in 0.1X SSC and 0.1% sodium dodecyl sulfate at 65°C.

By "encoding" or "encoded", with respect to a specified nucleic acid, is meant comprising the information for translation into the specified protein. A nucleic acid encoding a protein may comprise non-translated sequences (e.g., introns) within translated regions of the nucleic acid, or may lack such intervening non-translated sequences (e.g., as in cDNA). The information by which a protein is encoded is specified by the use of codons. Typically, the amino acid sequence is encoded by the nucleic acid using the "universal" genetic code. However, variants of the universal code, such as is present in some plant, animal, and fungal mitochondria, the bacterium *Mycoplasma capricolum* (*Proc. Natl. Acad. Sci. (USA)*, 82: 2306-2309 (1985)), or the ciliate *Macronucleus*, may be used when the nucleic acid is expressed using these organisms.

When the nucleic acid is prepared or altered synthetically, advantage can be taken of known codon preferences of the intended host where the nucleic acid is to be expressed. For example, although nucleic acid sequences of the present invention may be expressed in both monocotyledonous and dicotyledonous plant species, sequences can be modified to account for the specific codon preferences and GC content preferences of monocotyledonous plants or dicotyledonous plants as these preferences have been shown to differ (Murray *et al.* Nucl. Acids Res. 17: 477-498 (1989) and herein incorporated by reference). Thus, the maize preferred codon for a particular amino acid might be derived from known gene sequences from maize. Maize codon usage for 28 genes from maize plants is listed in Table 4 of Murray *et al., supra.*

As used herein, "heterologous" in reference to a nucleic acid is a nucleic acid that originates from a foreign species, or, if from the same species, is substantially modified from its native form in composition and/or genomic locus by deliberate human intervention. For example, a promoter operably linked to a heterologous structural gene is from a species different from that from which the structural gene was derived, or, if from the same species, one or both are substantially modified from their original form. A heterologous protein may originate from a foreign species or, if from the same species, is substantially modified from its original form by deliberate human intervention.

By "host cell" or “recombinantly engineered cell” is meant a cell, which contains a vector and supports the replication and/or expression of the expression vector. Host cells

may be prokaryotic cells such as *E. coli*, or eukaryotic cells such as yeast, *Pichia*, insect, plant, amphibian, or mammalian cells. Preferably, host cells are monocotyledonous or dicotyledonous plant cells, including but not limited to maize, sorghum, sunflower, soybean, wheat, alfalfa, rice, cotton, canola, barley, millet, and tomato. A particularly preferred monocotyledonous host cell is a maize host cell.

The term "hybridization complex" includes reference to a duplex nucleic acid structure formed by two single-stranded nucleic acid sequences selectively hybridized with each other.

The term “introduced” in the context of inserting a nucleic acid into a cell, means "transfection" or “transformation” or “transduction” and includes reference to the incorporation of a nucleic acid into a eukaryotic or prokaryotic cell where the nucleic acid may be incorporated into the genome of the cell (e.g., chromosome, plasmid, plastid or mitochondrial DNA), converted into an autonomous replicon, or transiently expressed (e.g., transfected mRNA).

The term "isolated" refers to material, such as a nucleic acid or a protein, which is substantially or essentially free from components which normally accompany or interact with it as found in its naturally occurring environment. The isolated material optionally comprises material not found with the material in its natural environment. Nucleic acids, which are “isolated”, as defined herein, are also referred to as “heterologous” nucleic acids.

Unless otherwise stated, the term “APAO nucleic acid” means a nucleic acid comprising a polynucleotide (“APAO polynucleotide”) encoding an APAO polypeptide. The term APAO, unless otherwise stated can encompass both APAO and the functional, truncated version of APAO designated trAPAO.

As used herein, "nucleic acid" includes reference to a deoxyribonucleotide or ribonucleotide polymer in either single- or double-stranded form, and unless otherwise limited, encompasses known analogues having the essential nature of natural nucleotides in that they hybridize to single-stranded nucleic acids in a manner similar to naturally occurring nucleotides (e.g., peptide nucleic acids).

By "nucleic acid library" is meant a collection of isolated DNA or RNA molecules, which comprise and substantially represent the entire transcribed fraction of a genome of a specified organism. Construction of exemplary nucleic acid libraries, such as genomic and cDNA libraries, is taught in standard molecular biology references such as Berger and

Kimmel, *Guide to Molecular Cloning Techniques, Methods in Enzymology,* Vol. 152, Academic Press, Inc., San Diego, CA (Berger); Sambrook *et al., Molecular Cloning - A Laboratory Manual,* 2nd ed., Vol. 1-3 (1989); and *Current Protocols in Molecular Biology*, F.M. Ausubel *et al.*, Eds., Current Protocols, a joint venture between Greene Publishing Associates, Inc. and John Wiley & Sons, Inc. (1994 Supplement).

As used herein "operably linked" includes reference to a functional linkage between a promoter and a second sequence, wherein the promoter sequence initiates and mediates transcription of the DNA sequence corresponding to the second sequence. Generally, operably linked means that the nucleic acid sequences being linked are contiguous and, where necessary to join two protein coding regions, contiguous and in the same reading frame.

The term "ligated" or "ligation" refers to the process of forming phosphodiester bonds between two or more polynucleotides, which most often are double stranded DNAs. Techniques for ligation are well known in the art and protocols are described in standard laboratory manuals and references, such as, Sambrook, *et al. Molecular Cloning: A Laboratory Manual*, 2ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York (1989). The two polynucleotides can include, but are not limited to, a polynucleotide, which can function as a promoter, ligated to a polynucleotide capable of encoding a polypeptide or linking two polynucleotides each capable of encoding a polypeptide. In the case of joining two polynucleotides that each encode a polypeptide, a polynucleotide spacer region between the two polynucleotides may or may not be present. The spacer region may encode a polypeptide containing a protease cleavage site. Optionally, the spacer region may contain a polynucleotide cleavage site such as but not limited to a site for RNAse cleavage or a self-cleaving ribozyme (*See, e.g.,* Tanner, *FEMS Microbiol Rev*, 23(3):257-75 (1999)). Alternatively, the transcription of the two or more ligated polynucleotides may result in a polycistronic message. An example of a spacer sequence that would direct translation of downstream coding sequences is an intervening ribosomal entry site (IRES) (*See, e.g.,* Liu, *et al., Anal Biochem*, 280(1):20-28 (2000)). The length of the spacer region may be of any length that results in a functional polypeptide or polypeptides. For example, the spacer region may be from 1 nucleotide to 1000 nucleotides, preferably 24 nucleotides in length.

As used herein, the term "plant" includes reference to whole plants, plant organs (e.g., leaves, stems, roots, etc.), seeds and plant cells and progeny of same. Plant cell, as used herein includes, without limitation, seeds suspension cultures, embryos, meristematic regions, callus tissue, leaves, roots, shoots, gametophytes, sporophytes, pollen, and microspores. The class of plants, which can be used in the methods of the invention, is generally as broad as the class of higher plants amenable to transformation techniques, including both monocotyledonous and dicotyledonous plants including species from the genera: *Cucurbita, Rosa, Vitis, Juglans, Fragaria, Lotus, Medicago, Onobrychis, Trifolium, Trigonella, Vigna, Citrus, Linum, Geranium, Manihot, Daucus, Arabidopsis, Brassica, Raphanus, Sinapis, Atropa, Capsicum, Datura, Hyoscyamus, Lycopersicon, Nicotiana, Solanum, Petunia, Digitalis, Majorana, Ciahorium, Helianthus, Lactuca, Bromus, Asparagus, Antirrhinum, Heterocallis, Nemesis, Pelargonium, Panieum, Pennisetum, Ranunculus, Senecio, Salpiglossis, Cucumis, Browaalia, Glycine, Pisum, Phaseolus, Lolium, Oryza, Avena, Hordeum, Secale, Allium,* and *Triticum*. A particularly preferred plant is *Zea mays*.

As used herein, “polynucleotide" includes reference to a deoxyribopolynucleotide, ribopolynucleotide, or analogs thereof that have the essential nature of a natural ribonucleotide in that they hybridize, under stringent hybridization conditions, to substantially the same nucleotide sequence as naturally occurring nucleotides and/or allow translation into the same amino acid(s) as the naturally occurring nucleotide(s). A polynucleotide can be full-length or a subsequence of a native or heterologous structural or regulatory gene. Unless otherwise indicated, the term includes reference to the specified sequence as well as the complementary sequence thereof. Thus, DNAs or RNAs with backbones modified for stability or for other reasons are "polynucleotides" as that term is intended herein. Moreover, DNAs or RNAs comprising unusual bases, such as inosine, or modified bases, such as tritylated bases, to name just two examples, are polynucleotides as the term is used herein. It will be appreciated that a great variety of modifications have been made to DNA and RNA that serve many useful purposes known to those of skill in the art. The term polynucleotide as it is employed herein embraces such chemically, enzymatically or metabolically modified forms of polynucleotides, as well as the chemical forms of DNA and RNA characteristic of viruses and cells, including *inter alia,* simple and complex cells.

The terms "polypeptide", "peptide" and "protein" are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical analogue of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers.

As used herein "promoter" includes reference to a region of DNA upstream from the start of transcription and involved in recognition and binding of RNA polymerase and other proteins to initiate transcription. A "plant promoter" is a promoter capable of initiating transcription in plant cells. Exemplary plant promoters include, but are not limited to, those that are obtained from plants, plant viruses, and bacteria which comprise genes expressed in plant cells such *Agrobacterium* or *Rhizobium*. Examples are promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, seeds, fibres, xylem vessels, tracheids, or sclerenchyma. Such promoters are referred to as "tissue preferred". A "cell type" specific promoter primarily drives expression in certain cell types in one or more organs, for example, vascular cells in roots or leaves. An “inducible” or “regulatable” promoter is a promoter, which is under environmental control. Examples of environmental conditions that may effect transcription by inducible promoters include anaerobic conditions or the presence of light. Another type of promoter is a developmentally regulated promoter, for example, a promoter that drives expression during pollen development. Tissue preferred, cell type specific, developmentally regulated, and inducible promoters constitute the class of "non-constitutive" promoters. A "constitutive" promoter is a promoter, which is active under most environmental conditions.

The term “APAO polypeptide or trAPAO polypeptide” refers to one or more amino acid sequences. The term is also inclusive of fragments, variants, homologs, alleles or precursors (e.g., preproproteins or proproteins) thereof. An “APAO or trAPAO protein” comprises an APAO or trAPAO polypeptide.

As used herein "recombinant" includes reference to a cell or vector, that has been modified by the introduction of a heterologous nucleic acid or that the cell is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found in identical form within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all as a result of deliberate human intervention. The term “recombinant” as used herein does not encompass the alteration of the cell or vector by naturally occurring events (e.g.,

spontaneous mutation, natural transformation/transduction/transposition) such as those occurring without deliberate human intervention.

As used herein, a "recombinant expression cassette" is a nucleic acid construct, generated recombinantly or synthetically, with a series of specified nucleic acid elements, which permit transcription of a particular nucleic acid in a target cell. The recombinant expression cassette can be incorporated into a plasmid, chromosome, mitochondrial DNA, plastid DNA, virus, or nucleic acid fragment. Typically, the recombinant expression cassette portion of an expression vector includes, among other sequences, a nucleic acid to be transcribed, and a promoter.

The term "residue" or "amino acid residue" or "amino acid" are used interchangeably herein to refer to an amino acid that is incorporated into a protein, polypeptide, or peptide (collectively "protein"). The amino acid may be a naturally occurring amino acid and, unless otherwise limited, may encompass known analogs of natural amino acids that can function in a similar manner as naturally occurring amino acids.

The term "selectively hybridizes" includes reference to hybridization, under stringent hybridization conditions, of a nucleic acid sequence to a specified nucleic acid target sequence to a detectably greater degree (e.g., at least 2-fold over background) than its hybridization to non-target nucleic acid sequences and to the substantial exclusion of non-target nucleic acids. Selectively hybridizing sequences typically have about at least 40% sequence identity, preferably 60-90% sequence identity, and most preferably 100% sequence identity (i.e., complementary) with each other.

The terms “stringent conditions” or “stringent hybridization conditions” include reference to conditions under which a probe will hybridize to its target sequence, to a detectably greater degree than other sequences (e.g., at least 2-fold over background). Stringent conditions are sequence-dependent and will be different in different circumstances. By controlling the stringency of the hybridization and/or washing conditions, target sequences can be identified which can be up to 100% complementary to the probe (homologous probing). Alternatively, stringency conditions can be adjusted to allow some mismatching in sequences so that lower degrees of similarity are detected (heterologous probing). Optimally, the probe is approximately 500 nucleotides in length,

but can vary greatly in length from less than 500 nucleotides to equal to the entire length of the target sequence.

Typically, stringent conditions will be those in which the salt concentration is less than about 1.5 M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3 and the temperature is at least about 30°C for short probes (*e.g.*, 10 to 50 nucleotides) and at least about 60°C for long probes (*e.g.*, greater than 50 nucleotides). Stringent conditions may also be achieved with the addition of destabilizing agents such as formamide or Denhardt's. Exemplary low stringency conditions include hybridization with a buffer solution of 30 to 35% formamide, 1 M NaCl, 1% SDS (sodium dodecyl sulphate) at 37°C, and a wash in 1X to 2X SSC (20X SSC = 3.0 M NaCl/0.3 M trisodium citrate) at 50 to 55°C. Exemplary moderate stringency conditions include hybridization in 40 to 45% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.5X to 1X SSC at 55 to 60°C. Exemplary high stringency conditions include hybridization in 50% formamide, 1 M NaCl, 1% SDS at 37°C, and a wash in 0.1X SSC at 60 to 65°C. Specificity is typically the function of post-hybridization washes, the critical factors being the ionic strength and temperature of the final wash solution. For DNA-DNA hybrids, the T_m can be approximated from the equation of Meinkoth and Wahl, *Anal. Biochem.*, 138:267-284 (1984): $T_m = 81.5\ °C + 16.6 (\log M) + 0.41 (\%GC) - 0.61 (\% \text{form}) - 500/L$; where M is the molarity of monovalent cations, %GC is the percentage of guanosine and cytosine nucleotides in the DNA, % form is the percentage of formamide in the hybridization solution, and L is the length of the hybrid in base pairs. The T_m is the temperature (under defined ionic strength and pH) at which 50% of a complementary target sequence hybridizes to a perfectly matched probe. T_m is reduced by about 1 °C for each 1% of mismatching; thus, T_m, hybridization and/or wash conditions can be adjusted to hybridize to sequences of the desired identity. For example, if sequences with $\geq$90% identity are sought, the T_m can be decreased 10 °C. Generally, stringent conditions are selected to be about 5 °C lower than the thermal melting point (T_m) for the specific sequence and its complement at a defined ionic strength and pH. However, severely stringent conditions can utilize a hybridization and/or wash at 1, 2, 3, or 4 °C lower than the thermal melting point (T_m); moderately stringent conditions can utilize a hybridization and/or wash at 6, 7, 8, 9, or 10 °C lower than the thermal melting point (T_m); low stringency conditions can utilize a hybridization and/or wash at 11, 12, 13, 14, 15, or 20 °C

lower than the thermal melting point (T_m). Using the equation, hybridization and wash compositions, and desired T_m, those of ordinary skill will understand that variations in the stringency of hybridization and/or wash solutions are inherently described. If the desired degree of mismatching results in a T_m of less than 45 °C (aqueous solution) or 32 °C (formamide solution) it is preferred to increase the SSC concentration so that a higher temperature can be used. An extensive guide to the hybridization of nucleic acids is found in Tijssen, *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes,* Part I, Chapter 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays", Elsevier, New York (1993); and *Current Protocols in Molecular Biology*, Chapter 2, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995). Unless otherwise stated, in the present application high stringency is defined as hybridization in 4X SSC, 5X Denhardt's (5g Ficoll, 5g polyvinypyrrolidone, 5 g bovine serum albumin in 500ml of water), 0.1 mg/ml boiled salmon sperm DNA, and 25 mM Na phosphate at 65°C, and a wash in 0.1X SSC, 0.1% SDS at 65°C.

As used herein, "transgenic plant" includes reference to a plant, which comprises within its genome a heterologous polynucleotide. Generally, the heterologous polynucleotide is stably integrated within the genome such that the polynucleotide is passed on to successive generations. The heterologous polynucleotide may be integrated into the genome alone or as part of a recombinant expression cassette. "Transgenic" is used herein to include any cell, cell line, callus, tissue, plant part or plant, the genotype of which has been altered by the presence of heterologous nucleic acid including those transgenics initially so altered as well as those created by sexual crosses or asexual propagation from the initial transgenic. The term "transgenic" as used herein does not encompass the alteration of the genome (chromosomal or extra-chromosomal) by conventional plant breeding methods or by naturally occurring events such as random cross-fertilization, non-recombinant viral infection, non-recombinant bacterial transformation, non-recombinant transposition, or spontaneous mutation.

As used herein, "vector" includes reference to a nucleic acid used in transfection of a host cell and into which can be inserted a polynucleotide. Vectors are often replicons. Expression vectors permit transcription of a nucleic acid inserted therein.

The following terms are used to describe the sequence relationships between two or more nucleic acids or polynucleotides or polypeptides: (a) "reference sequence", (b) "comparison window", (c) "sequence identity", (d) "percentage of sequence identity", and (e) "substantial identity".

(a) As used herein, "reference sequence" is a defined sequence used as a basis for sequence comparison. A reference sequence may be a subset or the entirety of a specified sequence; for example, as a segment of a full-length cDNA or gene sequence, or the complete cDNA or gene sequence.

(b) As used herein, "comparison window" means includes reference to a contiguous and specified segment of a polynucleotide sequence, wherein the polynucleotide sequence may be compared to a reference sequence and wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. Generally, the comparison window is at least 20 contiguous nucleotides in length, and optionally can be 30, 40, 50, 100, or longer. Those of skill in the art understand that to avoid a high similarity to a reference sequence due to inclusion of gaps in the polynucleotide sequence a gap penalty is typically introduced and is subtracted from the number of matches.

Methods of alignment of nucleotide and amino acid sequences for comparison are well known in the art. The local homology algorithm (Best Fit) of Smith and Waterman, Adv. Appl. Math may conduct optimal alignment of sequences for comparison. 2: 482 (1981); by the homology alignment algorithm (GAP) of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970); by the search for similarity method (Tfasta and Fasta) of Pearson and Lipman, *Proc. Natl. Acad. Sci.* 85: 2444 (1988); by computerized implementations of these algorithms, including, but not limited to: CLUSTAL in the PC/Gene program by Intelligenetics, Mountain View, California, GAP, BESTFIT, BLAST, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group (GCG), 575 Science Dr., Madison, Wisconsin, USA; the CLUSTAL program is well described by Higgins and Sharp, *Gene* 73: 237-244 (1988); Higgins and Sharp, *CABIOS* 5: 151-153 (1989); Corpet, *et al., Nucleic Acids Research* 16: 10881-90 (1988); Huang, *et al., Computer Applications in the Biosciences* 8: 155-65 (1992), and Pearson, *et al., Methods in Molecular Biology* 24: 307-331 (1994). The preferred program to use for optimal

global alignment of multiple sequences is PileUp (Feng and Doolittle, *Journal of Molecular Evolution*, 25:351-360 (1987) which is similar to the method described by Higgins and Sharp, *CABIOS*, 5:151-153 (1989) and hereby incorporated by reference). The BLAST family of programs which can be used for database similarity searches includes: BLASTN for nucleotide query sequences against nucleotide database sequences; BLASTX for nucleotide query sequences against protein database sequences; BLASTP for protein query sequences against protein database sequences; TBLASTN for protein query sequences against nucleotide database sequences; and TBLASTX for nucleotide query sequences against nucleotide database sequences. See, *Current Protocols in Molecular Biology*, Chapter 19, Ausubel, *et al.*, Eds., Greene Publishing and Wiley-Interscience, New York (1995).

GAP uses the algorithm of Needleman and Wunsch (J. Mol. Biol. 48: 443-453, 1970) to find the alignment of two complete sequences that maximizes the number of matches and minimizes the number of gaps. GAP considers all possible alignments and gap positions and creates the alignment with the largest number of matched bases and the fewest gaps. It allows for the provision of a gap creation penalty and a gap extension penalty in units of matched bases. GAP must make a profit of gap creation penalty number of matches for each gap it inserts. If a gap extension penalty greater than zero is chosen, GAP must, in addition, make a profit for each gap inserted of the length of the gap times the gap extension penalty. Default gap creation penalty values and gap extension penalty values in Version 10 of the Wisconsin Genetics Software Package are 8 and 2, respectively. The gap creation and gap extension penalties can be expressed as an integer selected from the group of integers consisting of from 0 to 100. Thus, for example, the gap creation and gap extension penalties can be 0, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 40, 50, or greater.

GAP presents one member of the family of best alignments. There may be many members of this family, but no other member has a better quality. GAP displays four figures of merit for alignments: Quality, Ratio, Identity, and Similarity. The Quality is the metric maximized in order to align the sequences. Ratio is the quality divided by the number of bases in the shorter segment. Percent Identity is the percent of the symbols that actually match. Percent Similarity is the percent of the symbols that are similar. Symbols that are across from gaps are ignored. A similarity is scored when the scoring matrix value

for a pair of symbols is greater than or equal to 0.50, the similarity threshold. The scoring matrix used in Version 10 of the Wisconsin Genetics Software Package is BLOSUM62 (*see* Henikoff & Henikoff (1989) *Proc. Natl. Acad. Sci. USA* 89:10915).

Unless otherwise stated, sequence identity/similarity values provided herein refer to the value obtained using the BLAST 2.0 suite of programs using default parameters. Altschul *et al.*, *Nucleic Acids Res.* 25:3389-3402 (1997).

As those of ordinary skill in the art will understand, BLAST searches assume that proteins can be modeled as random sequences. However, many real proteins comprise regions of nonrandom sequences, which may be homopolymeric tracts, short-period repeats, or regions enriched in one or more amino acids. Such low-complexity regions may be aligned between unrelated proteins even though other regions of the protein are entirely dissimilar. A number of low-complexity filter programs can be employed to reduce such low-complexity alignments. For example, the SEG (Wooten and Federhen, *Comput. Chem.*, 17:149-163 (1993)) and XNU (Claverie and States, *Comput. Chem.*, 17:191-201 (1993)) low-complexity filters can be employed alone or in combination.

(c) As used herein, "sequence identity" or "identity" in the context of two nucleic acid or polypeptide sequences includes reference to the residues in the two sequences, which are the same when aligned for maximum correspondence over a specified comparison window. When percentage of sequence identity is used in reference to proteins it is recognized that residue positions which are not identical often differ by conservative amino acid substitutions, where amino acid residues are substituted for other amino acid residues with similar chemical properties (e.g. charge or hydrophobicity) and therefore do not change the functional properties of the molecule. Where sequences differ in conservative substitutions, the percent sequence identity may be adjusted upwards to correct for the conservative nature of the substitution. Sequences, which differ by such conservative substitutions, are said to have "sequence similarity" or "similarity". Means for making this adjustment are well known to those of skill in the art. Typically this involves scoring a conservative substitution as a partial rather than a full mismatch, thereby increasing the percentage sequence identity. Thus, for example, where an identical amino acid is given a score of 1 and a non-conservative substitution is given a score of zero, a conservative substitution is given a score between zero and 1. The scoring of conservative substitutions is calculated, *e.g.*, according to the algorithm of Meyers and

Miller, *Computer Applic. Biol. Sci.*, 4: 11-17 (1988) *e.g.*, as implemented in the program PC/GENE (Intelligenetics, Mountain View, California, USA).

(d) As used herein, "percentage of sequence identity" means the value determined by comparing two optimally aligned sequences over a comparison window, wherein the portion of the polynucleotide sequence in the comparison window may comprise additions or deletions (i.e., gaps) as compared to the reference sequence (which does not comprise additions or deletions) for optimal alignment of the two sequences. The percentage is calculated by determining the number of positions at which the identical nucleic acid base or amino acid residue occurs in both sequences to yield the number of matched positions, dividing the number of matched positions by the total number of positions in the window of comparison and multiplying the result by 100 to yield the percentage of sequence identity.

(e) (i) The term "substantial identity" of polynucleotide sequences means that a polynucleotide comprises a sequence that has between 50-100% sequence identity, preferably at least 50% sequence identity, preferably at least 60% sequence identity, preferably at least 70%, more preferably at least 80%, more preferably at least 90% and most preferably at least 95%, compared to a reference sequence using one of the alignment programs described using standard parameters. One of skill will recognize that these values can be appropriately adjusted to determine corresponding identity of proteins encoded by two nucleotide sequences by taking into account codon degeneracy, amino acid similarity, reading frame positioning and the like. Substantial identity of amino acid sequences for these purposes normally means sequence identity of between 40-100%, preferably at least 55%, preferably at least 60%, more preferably at least 70%, 80%, 90%, and most preferably at least 95%.

Another indication that nucleotide sequences are substantially identical is if two molecules hybridize to each other under stringent conditions. The degeneracy of the genetic code allows for many amino acids substitutions that lead to variety in the nucleotide sequence that code for the same amino acid, hence it is possible that the DNA sequence could code for the same polypeptide but not hybridize to each other under stringent conditions. This may occur, *e.g.*, when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code. One indication that two nucleic acid sequences are substantially identical is that the polypeptide, which the first

nucleic acid encodes, is immunologically cross reactive with the polypeptide encoded by the second nucleic acid.

(e) (ii) The terms "substantial identity" in the context of a peptide indicates that a peptide comprises a sequence with between 55-100% sequence identity to a reference sequence preferably at least 55% sequence identity, preferably 60% preferably 70%, more preferably 80%, most preferably at least 90% or 95% sequence identity to the reference sequence over a specified comparison window. Preferably, optimal alignment is conducted using the homology alignment algorithm of Needleman and Wunsch, *J. Mol. Biol.* 48: 443 (1970). An indication that two peptide sequences are substantially identical is that one peptide is immunologically reactive with antibodies raised against the second peptide. Thus, a peptide is substantially identical to a second peptide, for example, where the two peptides differ only by a conservative substitution. In addition, a peptide can be substantially identical to a second peptide when they differ by a non-conservative change if the epitope that the antibody recognizes is substantially identical. Peptides, which are "substantially similar" share sequences as, noted above except that residue positions, which are not identical, may differ by conservative amino acid changes.

Fumonisin Degrading Organisms

The present invention is based on the discovery of organisms with the ability to degrade the mycotoxin fumonisin. In a search for a biological means of detoxifying fumonisins, several dematiaceous hyphomycetes were isolated from field-grown maize kernels. The fungi were found to be capable of growing on fumonisin B1 or B2 (FB1 or FB2) as a sole carbon source, degrading it partially or completely in the process. One species, identified as *Exophiala spinifera*, a "black yeast", was recovered from maize seed from diverse locations in the southeastern and south central US. The enzyme-active strain of *Exophiala spinifera* (ATCC 74269) was deposited (see US patent no. 5,716,820, issued February 10, 1998, US patent no. 5,792,931, issued August 11, 1998; US patent no. 6,025,188, issued February 15, 2000; and pending US application no. 08/888,950, filed July 7, 1997). Other enzyme-active strains of *Exophiala spinifera* were used to isolate APAO polynucleotides. Isolate ESP002 was isolated from palm trees (ATCC 26089) and isolate ESP003 was isolated from maize seed. Another fungus from which APAO polynucleotides were isolated was *Rhinocladiella atrovirens* (RAT 011).

Nucleic Acids

The present invention provides, *inter alia*, isolated nucleic acids of RNA, DNA, and analogs and/or chimeras thereof, comprising an APAO or trAPAO polynucleotide.

The present invention also includes polynucleotides optimized for expression in different organisms. For example, for expression of the polynucleotide in a maize plant, the sequence can be altered to account for specific codon preferences and to alter GC content as according to Murray *et al*, *supra*. Maize codon usage for 28 genes from maize plants is listed in Table 4 of Murray, *et al., supra.*

The APAO or trAPAO nucleic acids of the present invention comprise isolated APAO or trAPAO polynucleotides which, are inclusive of:

(a) a polynucleotide encoding an APAO or trAPAO polypeptide of the sequences shown in SEQ ID NOS: 36, 38, 40, 42, 44, and 46, and conservatively modified and polymorphic variants thereof;

(b) a polynucleotide which selectively hybridizes to a polynucleotide of (a) or (b);

(c) a polynucleotide having at least 50% sequence identity with polynucleotides of (a) or (b);

(d) complementary sequences of polynucleotides of (a), (b), or (c); and

(e) a polynucleotide comprising at least 25 contiguous nucleotides from a polynucleotide of (a), (b), (c), or (d).

In addition, polynucleotides are presented that are a fusion of an APAO or trAPAO polynucleotide and the polynucleotide of a fumonisin esterase. The invention encompasses the sequences from *Exophiala* or *Rhinocladiella* as well as sequences having sequence similarity with such sequences. It is recognized that the sequences of the invention can be used to isolate corresponding sequences in other organisms. Methods such as PCR, hybridization, and the like can be used to identify sequences having substantial sequence similarity to the sequences of the invention. See, for example, Sambrook, *et al.,* (1989) *Molecular Cloning: A Laboratory Manual* (2d ed., Cold Spring Harbor Laboratory Press, Planview, New York) and Innis *et al.,* (1990) *PCR Protocols: Guide to Methods and Applications* (Academic Press, New York). Coding sequences isolated based on their sequence identity to the entire fumonisin degrading coding

sequences set forth herein or to fragments thereof are encompassed by the present invention.

It is recognized that the sequences of the invention can be used to isolate similar sequences from other fumonisin degrading organisms. Likewise sequences from other fumonisin degrading organisms may be used in combination with the sequences of the present invention. See, for example, copending application entitled "Compositions and Methods for Fumonisin Detoxification", U.S. application serial number 60/092,953, filed concurrently herewith and herein incorporated by reference.

Plasmids containing the polynucleotide sequences of the invention were deposited with American Type Culture Collection (ATCC), Manassas, Virginia, and assigned Accession Nos. 98812, 98813, 98814, 98815, 98816, and PTA-32. These deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. These deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. § 112.

Construction of Nucleic Acids

The isolated nucleic acids of the present invention can be made using (a) standard recombinant methods, (b) synthetic techniques, or combinations thereof. In some embodiments, the polynucleotides of the present invention will be cloned, amplified, or otherwise constructed from a fungus or bacteria.

The nucleic acids may conveniently comprise sequences in addition to a polynucleotide of the present invention. For example, a multi-cloning site comprising one or more endonuclease restriction sites may be inserted into the nucleic acid to aid in isolation of the polynucleotide. Also, translatable sequences may be inserted to aid in the isolation of the translated polynucleotide of the present invention. For example, a hexa-histidine marker sequence provides a convenient means to purify the proteins of the present invention. The nucleic acid of the present invention - excluding the polynucleotide sequence - is optionally a vector, adapter, or linker for cloning and/or expression of a polynucleotide of the present invention. Additional sequences may be added to such cloning and/or expression sequences to optimize their function in cloning and/or expression, to aid in isolation of the polynucleotide, or to improve the introduction of the

polynucleotide into a cell. Typically, the length of a nucleic acid of the present invention less the length of its polynucleotide of the present invention is less than 20 kilobase pairs, often less than 15 kb, and frequently less than 10 kb. Use of cloning vectors, expression vectors, adapters, and linkers is well known in the art. Exemplary nucleic acids include such vectors as: M13, lambda ZAP Express, lambda ZAP II, lambda gt10, lambda gt11, pBK-CMV, pBK-RSV, pBluescript II, lambda DASH II, lambda EMBL 3, lambda EMBL 4, pWE15, SuperCos 1, SurfZap, Uni-ZAP, pBC, pBS+/-, pSG5, pBK, pCR-Script, pET, pSPUTK, p3'SS, pGEM, pSK+/-, pGEX, pSPORTI and II, pOPRSVI CAT, pOPI3 CAT, pXT1, pSG5, pPbac, pMbac, pMC1neo, pOG44, pOG45, pFRTβGAL, pNEOβGAL, pRS403, pRS404, pRS405, pRS406, pRS413, pRS414, pRS415, pRS416, lambda MOSSlox, and lambda MOSElox. Optional vectors for the present invention, include but are not limited to, lambda ZAP II, and pGEX. For a description of various nucleic acids see, for example, Stratagene Cloning Systems, Catalogs 1995, 1996, 1997 (La Jolla, CA); and, Amersham Life Sciences, Inc, Catalog '97 (Arlington Heights, IL).

Synthetic Methods for Constructing Nucleic Acids

The isolated nucleic acids of the present invention can also be prepared by direct chemical synthesis by methods such as the phosphotriester method of Narang *et al., Meth. Enzymol.* 68: 90-99 (1979); the phosphodiester method of Brown *et al., Meth. Enzymol.* 68: 109-151 (1979); the diethylphosphoramidite method of Beaucage *et al., Tetra. Lett.* 22: 1859-1862 (1981); the solid phase phosphoramidite triester method described by Beaucage and Caruthers, *Tetra. Letts.* 22(20): 1859-1862 (1981), *e.g.,* using an automated synthesizer, *e.g.,* as described in Needham-VanDevanter *et al., Nucleic Acids Res.*, 12: 6159-6168 (1984); and, the solid support method of US Patent No. 4,458,066. Chemical synthesis generally produces a single stranded oligonucleotide. This may be converted into double stranded DNA by hybridization with a complementary sequence, or by polymerization with a DNA polymerase using the single strand as a template. One of skill will recognize that while chemical synthesis of DNA is limited to sequences of about 100 bases, longer sequences may be obtained by the ligation of shorter sequences.

UTRs and Codon Preference

In general, translational efficiency has been found to be regulated by specific sequence elements in the 5' non-coding or untranslated region (5' UTR) of the RNA. Positive sequence motifs include translational initiation consensus sequences (Kozak, *Nucleic Acids Res.*15:8125 (1987)) and the 5<G> 7 methyl GpppG RNA cap structure (Drummond *et al., Nucleic Acids Res.* 13:7375 (1985)). Negative elements include stable intramolecular 5' UTR stem-loop structures (Muesing *et al., Cell* 48:691 (1987)) and AUG sequences or short open reading frames preceded by an appropriate AUG in the 5' UTR (Kozak, *supra, Rao et al., Mol. and Cell. Biol.* 8:284 (1988)). Accordingly, the present invention provides 5' and/or 3' UTR regions for modulation of translation of heterologous coding sequences.

Further, the polypeptide-encoding segments of the polynucleotides of the present invention can be modified to alter codon usage. Altered codon usage can be employed to alter translational efficiency and/or to optimize the coding sequence for expression in a desired host or to optimize the codon usage in a heterologous sequence for expression in maize. Codon usage in the coding regions of the polynucleotides of the present invention can be analyzed statistically using commercially available software packages such as "Codon Preference" available from the University of Wisconsin Genetics Computer Group (see Devereaux *et al., Nucleic Acids Res.* 12: 387-395 (1984)) or MacVector 4.1 (Eastman Kodak Co., New Haven, Conn.). Thus, the present invention provides a codon usage frequency characteristic of the coding region of at least one of the polynucleotides of the present invention. The number of polynucleotides (3 nucleotides per amino acid) that can be used to determine a codon usage frequency can be any integer from 3 to the number of polynucleotides of the present invention as provided herein. Optionally, the polynucleotides will be full-length sequences. An exemplary number of sequences for statistical analysis can be at least 1, 5, 10, 20, 50, or 100.

Sequence Shuffling

The present invention provides methods for sequence shuffling using polynucleotides of the present invention, and compositions resulting therefrom. Sequence shuffling is described in PCT publication No. 96/19256. See also, Zhang, J.- H., *et al. Proc. Natl. Acad. Sci. USA* 94:4504-4509 (1997) and Zhao, *et al., Nature Biotech* 16:258-

261 (1998). Generally, sequence shuffling provides a means for generating libraries of polynucleotides having a desired characteristic, which can be selected or screened for. Libraries of recombinant polynucleotides are generated from a population of related sequence polynucleotides , which comprise sequence regions, which have substantial sequence identity and can be homologously recombined in vitro or in vivo. The population of sequence-recombined polynucleotides comprises a subpopulation of polynucleotides which possess desired or advantageous characteristics and which can be selected by a suitable selection or screening method. The characteristics can be any property or attribute capable of being selected for or detected in a screening system, and may include properties of: an encoded protein, a transcriptional element, a sequence controlling transcription, RNA processing, RNA stability, chromatin conformation, translation, or other expression property of a gene or transgene, a replicative element, a protein-binding element, or the like, such as any feature which confers a selectable or detectable property. In some embodiments, the selected characteristic will be an altered K_m and/or K_{cat} over the wild-type protein as provided herein. In other embodiments, a protein or polynucleotide generated from sequence shuffling will have a substrate binding affinity greater than the non-shuffled wild-type polynucleotide. In yet other embodiments, a protein or polynucleotide generated from sequence shuffling will have an altered pH optimum as compared to the non-shuffled wild-type polynucleotide. The increase in such properties can be at least 110%, 120%, 130%, 140% or greater than 150% of the wild-type value.

Recombinant Expression Cassettes

The present invention further provides recombinant expression cassettes comprising a nucleic acid of the present invention. A nucleic acid sequence coding for the desired polynucleotide of the present invention, for example a cDNA or a genomic sequence encoding a polypeptide long enough to code for an active protein of the present invention, can be used to construct a recombinant expression cassette which can be introduced into the desired host cell. A recombinant expression cassette will typically comprise a polynucleotide of the present invention operably linked to transcriptional initiation regulatory sequences which will direct the transcription of the polynucleotide in the intended host cell, such as tissues of a transformed plant.

For example, plant expression vectors may include (1) a cloned plant gene under the transcriptional control of 5' and 3' regulatory sequences and (2) a dominant selectable marker. Such plant expression vectors may also contain, if desired, a promoter regulatory region (e.g., one conferring inducible or constitutive, environmentally- or developmentally-regulated, or cell- or tissue-specific/selective expression), a transcription initiation start site, a ribosome binding site, an RNA processing signal, a transcription termination site, and/or a polyadenylation signal.

A plant promoter fragment can be employed which will direct expression of a polynucleotide of the present invention in all tissues of a regenerated plant. Such promoters are referred to herein as "constitutive" promoters and are active under most environmental conditions and states of development or cell differentiation. Examples of constitutive promoters include the 1'- or 2'- promoter derived from T-DNA of *Agrobacterium tumefaciens*, the Smas promoter, the cinnamyl alcohol dehydrogenase promoter (US Patent No. 5,683,439), the *Nos* promoter, the rubisco promoter, the GRP1-8 promoter, the 35S promoter from cauliflower mosaic virus (CaMV), as described in Odell et al., (1985), *Nature*, 313:810-812, rice actin (McElroy et al., (1990), *Plant Cell*, 163-171); ubiquitin (Christensen et al., (1992), *Plant Mol. Biol.* 12:619-632; and Christensen, et al., (1992), *Plant Mol. Biol.* 18:675-689); pEMU (Last, et al., (1991), *Theor. Appl. Genet.* 81:581-588); MAS (Velten et al., (1984), *EMBO J.* 3:2723-2730); and maize H3 histone (Lepetit et al., (1992), *Mol. Gen. Genet.* 231:276-285; and Atanassvoa et al., (1992), *Plant Journal* 2(3):291-300), the Rsyn7 as described in published PCT Application WO 97/44756, ALS promoter, as described in published PCT Application WO 96/30530, and other transcription initiation regions from various plant genes known to those of skill. For the present invention ubiquitin is the preferred promoter for expression in monocot plants.

Alternatively, the plant promoter can direct expression of a polynucleotide of the present invention in a specific tissue or may be otherwise under more precise environmental or developmental control. Such promoters are referred to here as "inducible" promoters. Environmental conditions that may effect transcription by inducible promoters include pathogen attack, anaerobic conditions, or the presence of light. Examples of inducible promoters are the Adh1 promoter, which is inducible by hypoxia or

cold stress, the Hsp70 promoter, which is inducible by heat stress, and the PPDK promoter, which is inducible by light.

Examples of promoters under developmental control include promoters that initiate transcription only, or preferentially, in certain tissues, such as leaves, roots, fruit, seeds, or flowers. The operation of a promoter may also vary depending on its location in the genome. Thus, an inducible promoter may become fully or partially constitutive in certain locations.

If polypeptide expression is desired, it is generally desirable to include a polyadenylation region at the 3'-end of a polynucleotide coding region. The polyadenylation region can be derived from a variety of plant genes, or from T-DNA. The 3' end sequence to be added can be derived from, for example, the nopaline synthase or octopine synthase genes, or alternatively from another plant gene, or less preferably from any other eukaryotic gene. Examples of such regulatory elements include, but are not limited to, 3' termination and/or polyadenylation regions such as those of the *Agrobacterium tumefaciens* nopaline synthase (nos) gene (Bevan et al., (1983), *Nucl. Acids Res.* 12:369-385); the potato proteinase inhibitor II (PINII) gene (Keil, et al., (1986), *Nucl. Acids Res.* 14:5641-5650; and An et al., (1989), *Plant Cell* 1:115-122); and the CaMV 19S gene (Mogen et al., (1990), *Plant Cell* 2:1261-1272).

An intron sequence can be added to the 5' untranslated region or the coding sequence of the partial coding sequence to increase the amount of the mature message that accumulates in the cytosol. Inclusion of a spliceable intron in the transcription unit in both plant and animal expression constructs has been shown to increase gene expression at both the mRNA and protein levels up to 1000-fold. Buchman and Berg, *Mol. Cell Biol.* 8: 4395-4405 (1988); Callis *et al., Genes Dev.* 1: 1183-1200 (1987). Such intron enhancement of gene expression is typically greatest when placed near the 5' end of the transcription unit. Use of maize introns Adh1-S intron 1, 2, and 6, the Bronze-1 intron are known in the art. See generally, *The Maize Handbook*, Chapter 116, Freeling and Walbot, Eds., Springer, New York (1994).

Plant signal sequences, including, but not limited to, signal-peptide encoding DNA/RNA sequences which target proteins to the extracellular matrix of the plant cell (Dratewka-Kos, et al., (1989), *J. Biol. Chem.* 264:4896-4900), the *Nicotiana plumbaginifolia* extension gene (DeLoose, et al., (1991), *Gene* 99:95-100), signal peptides

which target proteins to the vacuole like the sweet potato sporamin gene (Matsuka, et al., (1991), *PNAS* 88:834) and the barley lectin gene (Wilkins, et al., (1990), *Plant Cell*, 2:301-313), signal peptides which cause proteins to be secreted such as that of PRIb (Lind, et al., (1992), *Plant Mol. Biol.* 18:47-53), or the barley alpha amylase (BAA) (Rahmatullah, *et al.*, *Plant Mol. Biol.* 12:119 (1989)) and hereby incorporated by reference), or from the present invention the signal peptide from the ESP1 or BEST1 gene, or signal peptides which target proteins to the plastids such as that of rapeseed enoyl-Acp reductase (Verwaert, et al., (1994), *Plant Mol. Biol.* 26:189-202) are useful in the invention. The barley alpha amylase signal sequence operably linked to the trAPAO or APAO polynucleotide is the preferred construct for expression in maize for the present invention.

The vector comprising the sequences from a polynucleotide of the present invention will typically comprise a marker gene, which confers a selectable phenotype on plant cells. Usually, the selectable marker gene will encode antibiotic resistance, with suitable genes including genes coding for resistance to the antibiotic spectinomycin (e.g., the aada gene), the streptomycin phosphotransferase (SPT) gene coding for streptomycin resistance, the neomycin phosphotransferase (NPTII) gene encoding kanamycin or geneticin resistance, the hygromycin phosphotransferase (HPT) gene coding for hygromycin resistance, genes coding for resistance to herbicides which act to inhibit the action of acetolactate synthase (ALS), in particular the sulfonylurea-type herbicides (e.g., the acetolactate synthase (ALS) gene containing mutations leading to such resistance in particular the S4 and/or Hra mutations), genes coding for resistance to herbicides which act to inhibit action of glutamine synthase, such as phosphinothricin or basta (e.g., the *bar* gene), or other such genes known in the art. The *bar* gene encodes resistance to the herbicide basta, and the ALS gene encodes resistance to the herbicide chlorsulfuron.

Alternatively, the invention, itself, could be used as a method for selection of transformants, in other words as a selectable marker. An APAO or trAPAO polynucleotide operably linked to a promoter and then transformed into a plant cell by any of the methods described in the present application would express the degradative enzyme. When the plant cells are placed in the presence of fumonisin, AP1, or a phytotoxic analog in culture only the transformed cells would be able to grow. In another embodiment, the plant cell could be transformed with both a polynucleotide for APAO and a polynucleotide for fumonisin esterase. The selective agent in this case could be either AP1 or fumonisin

or any structural analog. Thus, growth of plant cells in the presence of a mycotoxin favors the survival of plant cells that have been transformed to express the coding sequence that codes for one of the enzymes of this invention and degrades the toxin. When the APAO or trAPAO cassette with or without the fumonisin esterase polynucleotide, is co-transformed with another gene of interest and then placed in the presence of fumonisin, AP1 or a phytotoxic analog, this invention would allow for selection of only those plant cells that contain the gene of interest. In the past antibiotic resistance genes have been used as selectable markers. Given the current concerns by consumers and environmentalist over use of antibiotic genes and the possibility of resistant microorganisms arising due to this use, a non-antibiotic resistant selectable marker system such as the present invention, fulfills this very important need.

Typical vectors useful for expression of genes in higher plants are well known in the art and include vectors derived from the tumor-inducing (Ti) plasmid of *Agrobacterium tumefaciens* described by Rogers *et al.*, Meth. In Enzymol., 153:253-277 (1987). These vectors are plant integrating vectors in that on transformation, the vectors integrate a portion of vector DNA into the genome of the host plant. Exemplary *A. tumefaciens* vectors useful herein are plasmids pKYLX6 and pKYLX7 of Schardl *et al.*, Gene, 61:1-11 (1987) and Berger *et al.*, Proc. Natl. Acad. Sci. U.S.A., 86:8402-8406 (1989). Another useful vector herein is plasmid pBI101.2 that is available from CLONTECH Laboratories, Inc. (Palo Alto, CA).

Expression of Proteins in Host Cells

Using the nucleic acids of the present invention, one may express a protein of the present invention in a recombinantly engineered cell such as bacteria, yeast, insect, mammalian, or preferably plant cells. The cells produce the protein in a non-natural condition (e.g., in quantity, composition, location, and/or time), because they have been genetically altered through human intervention to do so.

It is expected that those of skill in the art are knowledgeable in the numerous expression systems available for expression of a nucleic acid encoding a protein of the present invention. No attempt to describe in detail the various methods known for the expression of proteins in prokaryotes or eukaryotes will be made.

In brief summary, the expression of isolated nucleic acids encoding a protein of the present invention will typically be achieved by operably linking, for example, the DNA or cDNA to a promoter (which is either constitutive or inducible), followed by incorporation into an expression vector. The vectors can be suitable for replication and integration in either prokaryotes or eukaryotes. Typical expression vectors contain transcription and translation terminators, initiation sequences, and promoters useful for regulation of the expression of the DNA encoding a protein of the present invention. To obtain high level expression of a cloned gene, it is desirable to construct expression vectors which contain, at the minimum, a strong promoter, such as ubiquitin, to direct transcription, a ribosome binding site for translational initiation, and a transcription/translation terminator. Constitutive promoters are classified as providing for a range of constitutive expression. Thus, some are weak constitutive promoters, and others are strong constitutive promoters. Generally, by “weak promoter” is intended a promoter that drives expression of a coding sequence at a low level. By “low level” is intended at levels of about 1/10,000 transcripts to about 1/100,000 transcripts to about 1/500,000 transcripts. Conversely, a “strong promoter” drives expression of a coding sequence at a “high level”, or about 1/10 transcripts to about 1/100 transcripts to about 1/1,000 transcripts.

One of skill would recognize that modifications could be made to a protein of the present invention without diminishing its biological activity. Some modifications may be made to facilitate the cloning, expression, or incorporation of the targeting molecule into a fusion protein. Such modifications are well known to those of skill in the art and include, for example, a methionine added at the amino terminus to provide an initiation site, or additional amino acids (*e.g.,* poly His) placed on either terminus to create conveniently located restriction sites or termination codons or purification sequences.

A. Expression in Prokaryotes

Prokaryotic cells may be used as hosts for expression. Prokaryotes most frequently are represented by various strains of *E. coli;* however, other microbial strains may also be used. Commonly used prokaryotic control sequences which are defined herein to include promoters for transcription initiation, optionally with an operator, along with ribosome binding site sequences, include such commonly used promoters as the beta lactamase (penicillinase) and lactose (lac) promoter systems (Chang et al., Nature 198:1056 (1977)),

the tryptophan (trp) promoter system (Goeddel et al., Nucleic Acids Res. 8:4057 (1980)) and the lambda derived P L promoter and N-gene ribosome binding site (Shimatake *et al.*, Nature 292:128 (1981)). The inclusion of selection markers in DNA vectors transfected in *E. coli* is also useful. Examples of such markers include genes specifying resistance to ampicillin, tetracycline, or chloramphenicol.

The vector is selected to allow introduction of the gene of interest into the appropriate host cell. Bacterial vectors are typically of plasmid or phage origin. Appropriate bacterial cells are infected with phage vector particles or transfected with naked phage vector DNA. If a plasmid vector is used, the bacterial cells are transfected with the plasmid vector DNA. Expression systems for expressing a protein of the present invention are available using *Bacillus sp.* and *Salmonella* (Palva, *et al.*, *Gene* 22: 229-235 (1983); Mosbach, *et al.*, *Nature* 302: 543-545 (1983)). The pGEX-4T-1 plasmid vector from Pharmacia is the preferred *E. coli* expression vector for the present invention.

B. Expression in Eukaryotes

A variety of eukaryotic expression systems such as yeast, insect cell lines, plant and mammalian cells, are known to those of skill in the art. As explained briefly below, the present invention can be expressed in these eukaryotic systems. In some embodiments, transformed/transfected plant cells, as discussed *infra*, are employed as expression systems for production of the proteins of the instant invention.

Synthesis of heterologous proteins in yeast is well known. Sherman, F., *et al.*, *Methods in Yeast Genetics*, Cold Spring Harbor Laboratory (1982) is a well recognized work describing the various methods available to produce the protein in yeast. Two widely utilized yeasts for production of eukaryotic proteins are *Saccharomyces cerevisiae* and *Pichia pastoris*. Vectors, strains, and protocols for expression in *Saccharomyces* and *Pichia* are known in the art and available from commercial suppliers (e.g., Invitrogen). Suitable vectors usually have expression control sequences, such as promoters, including 3-phosphoglycerate kinase or alcohol oxidase, and an origin of replication, termination sequences and the like as desired.

A protein of the present invention, once expressed, can be isolated from yeast by lysing the cells and applying standard protein isolation techniques to the lysates or the

pellets. The monitoring of the purification process can be accomplished by using Western blot techniques or radioimmunoassay of other standard immunoassay techniques.

The sequences encoding proteins of the present invention can also be ligated to various expression vectors for use in transfecting cell cultures of, for instance, mammalian, insect, or plant origin. Mammalian cell systems often will be in the form of monolayers of cells although mammalian cell suspensions may also be used. A number of suitable host cell lines capable of expressing intact proteins have been developed in the art, and include the HEK293, BHK21, and CHO cell lines. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter (*e.g.*, the CMV promoter, a HSV *tk* promoter or *pgk* (phosphoglycerate kinase) promoter), an enhancer (Queen *et al., Immunol. Rev.* 89: 49 (1986)), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites (*e.g.*, an SV40 large T Ag poly A addition site), and transcriptional terminator sequences. Other animal cells useful for production of proteins of the present invention are available, for instance, from the American Type Culture Collection Catalogue of Cell Lines and Hybridomas (7th edition, 1992).

Appropriate vectors for expressing proteins of the present invention in insect cells are usually derived from the SF9 baculovirus. Suitable insect cell lines include mosquito larvae, silkworm, armyworm, moth, and *Drosophila* cell lines such as a Schneider cell line (See Schneider, *J. Embryol. Exp. Morphol.* 27: 353-365 (1987).

As with yeast, when higher animal or plant host cells are employed, polyadenlyation or transcription terminator sequences are typically incorporated into the vector. An example of a terminator sequence is the polyadenlyation sequence from the bovine growth hormone gene. Sequences for accurate splicing of the transcript may also be included. An example of a splicing sequence is the VP1 intron from SV40 (Sprague, *et al.*, *J. Virol.* 45: 773-781 (1983)). Additionally, gene sequences to control replication in the host cell may be incorporated into the vector such as those found in bovine papilloma virus type-vectors. Saveria-Campo, M., Bovine Papilloma Virus DNA a Eukaryotic Cloning Vector in *DNA Cloning Vol. II a Practical Approach,* D.M. Glover, Ed., IRL Press, Arlington, Virginia pp. 213-238 (1985).

In addition, one of the genes for fumonisin esterase or the APAO or trAPAO placed in the appropriate plant expression vector can be used to transform plant cells. The

enzyme can then be isolated from plant callus or the transformed cells can be used to regenerate transgenic plants. Such transgenic plants can be harvested, and the appropriate tissues (seed or leaves, for example) can be subjected to large scale protein extraction and purification techniques, and the fumonisin degradation enzymes or APAO can be isolated for use in fumonisin and fumonisin hydrolysis product detoxification processes.

Plant Transformation Methods

Numerous methods for introducing foreign genes into plants are known and can be used to insert an APAO or trAPAO polynucleotide into a plant host, including biological and physical plant transformation protocols. See, for example, Miki et al., (1993), "Procedure for Introducing Foreign DNA into Plants", In: *Methods in Plant Molecular Biology and Biotechnology*, Glick and Thompson, eds., CRC Press, Inc., Boca Raton, pages 67-88. The methods chosen vary with the host plant, and include chemical transfection methods such as calcium phosphate, microorganism-mediated gene transfer such as *Agrobacterium* (Horsch, et al., (1985), *Science* 227:1229-31), electroporation, micro-injection, and biolistic bombardment.

Expression cassettes and vectors and *in vitro* culture methods for plant cell or tissue transformation and regeneration of plants are known and available. See, for example, Gruber, et al., (1993), "Vectors for Plant Transformation" In: *Methods in Plant Molecular Biology and Biotechnology*, Glick and Thompson, eds. CRC Press, Inc., Boca Raton, pages 89-119.

***Agrobacterium*-mediated Transformation**

The most widely utilized method for introducing an expression vector into plants is based on the natural transformation system of *Agrobacterium*. *A. tumefaciens* and *A. rhizogenes* are plant pathogenic soil bacteria, which genetically transform plant cells. The Ti and Ri plasmids of *A. tumefaciens* and *A. rhizogenes*, respectively, carry genes responsible for genetic transformation of plants. See, for example, Kado, (1991), *Crit. Rev. Plant Sci.* 10:1. Descriptions of the *Agrobacterium* vector systems and methods for *Agrobacterium*-mediated gene transfer are provided in Gruber et al., *supra*; Miki, et al., *supra*; and Moloney et al., (1989), *Plant Cell Reports* 8:238.

Similarly, the gene can be inserted into the T-DNA region of a Ti or Ri plasmid derived from *A. tumefaciens* or *A. rhizogenes*, respectively. Thus, expression cassettes can be constructed as above, using these plasmids. Many control sequences are known which when coupled to a heterologous coding sequence and transformed into a host organism show fidelity in gene expression with respect to tissue/organ specificity of the original coding sequence. See, e.g., Benfey, P. N., and Chua, N. H. (1989) *Science* 244: 174-181. Particularly suitable control sequences for use in these plasmids are promoters for constitutive leaf-specific expression of the gene in the various target plants. Other useful control sequences include a promoter and terminator from the nopaline synthase gene (NOS). The NOS promoter and terminator are present in the plasmid pARC2, available from the American Type Culture Collection and designated ATCC 67238. If such a system is used, the virulence (*vir*) gene from either the Ti or Ri plasmid must also be present, either along with the T-DNA portion, or via a binary system where the *vir* gene is present on a separate vector. Such systems, vectors for use therein, and methods of transforming plant cells are described in US Pat. No. 4,658,082; US application Ser. No. 913,914, filed Oct. 1, 1986, as referenced in US Patent 5,262,306, issued November 16, 1993 to Robeson, et al.; and Simpson, R. B., et al. (1986) *Plant Mol. Biol.* 6: 403-415 (also referenced in the '306 patent); all incorporated by reference in their entirety.

Once constructed, these plasmids can be placed into *A. rhizogenes* or *A. tumefaciens* and these vectors used to transform cells of plant species, which are ordinarily susceptible to *Fusarium* or *Alternaria* infection. Several other transgenic plants are also contemplated by the present invention including but not limited to soybean, corn, sorghum, alfalfa, rice, clover, cabbage, banana, coffee, celery, tobacco, cowpea, cotton, melon and pepper. The selection of either *A. tumefaciens* or *A. rhizogenes* will depend on the plant being transformed thereby. In general *A. tumefaciens* is the preferred organism for transformation. Most dicotyledonous plants, some gymnosperms, and a few monocotyledonous plants (e.g. certain members of the *Liliales* and *Arales*) are susceptible to infection with *A. tumefaciens*. *A. rhizogenes* also has a wide host range, embracing most dicots and some gymnosperms, which includes members of the *Leguminosae*, *Compositae*, and *Chenopodiaceae*. Monocot plants can now be transformed with some success. European Patent Application Publication Number 604 662 A1 to Hiei *et al.* discloses a method for transforming monocots using *Agrobacterium*. Saito *et al.* discloses a method

for transforming monocots with *Agrobacterium* using the scutellum of immature embryos (European Application 672 752 A1). Ishida *et al.* discusses a method for transforming maize by exposing immature embryos to *A. tumefaciens* (Ishida *et al., Nature Biotechnology*, 1996, 14:745-750).

Once transformed, these cells can be used to regenerate transgenic plants, capable of degrading fumonisin. For example, whole plants can be infected with these vectors by wounding the plant and then introducing the vector into the wound site. Any part of the plant can be wounded, including leaves, stems and roots. Alternatively, plant tissue, in the form of an explant, such as cotyledonary tissue or leaf disks, can be inoculated with these vectors, and cultured under conditions, which promote plant regeneration. Roots or shoots transformed by inoculation of plant tissue with *A. rhizogenes* or *A. tumefaciens*, containing the gene coding for the fumonisin degradation enzyme, can be used as a source of plant tissue to regenerate fumonisin-resistant transgenic plants, either via somatic embryogenesis or organogenesis. Examples of such methods for regenerating plant tissue are disclosed in Shahin, E. A. (1985) *Theor. Appl. Genet.* 69:235-240; US Pat. No. 4,658,082; Simpson, R. B., et al. (1986) *Plant Mol. Biol.* 6: 403-415; and U.S. patent applications Ser. Nos. 913,913 and 913,914, both filed Oct. 1, 1986, as referenced in U.S. Patent 5,262,306, issued November 16, 1993 to Robeson, et al.; the entire disclosures therein incorporated herein by reference.

Direct Gene Transfer

Despite the fact that the host range for *Agrobacterium*-mediated transformation is broad, some major cereal crop species and gymnosperms have generally been recalcitrant to this mode of gene transfer, even though some success has recently been achieved in rice (Hiei et al., (1994), *The Plant Journal* 6:271-282). Several methods of plant transformation, collectively referred to as direct gene transfer, have been developed as an alternative to *Agrobacterium*-mediated transformation.

A generally applicable method of plant transformation is microprojectile-mediated transformation, where DNA is carried on the surface of microprojectiles measuring about 1 to 4 μm. The expression vector is introduced into plant tissues with a biolistic device that accelerates the microprojectiles to speeds of 300 to 600 m/s which is sufficient to penetrate the plant cell walls and membranes. (Sanford et al., (1987), *Part. Sci. Technol.* 5:27;

Sanford, 1988, *Trends Biotech* 6:299; Sanford, (1990), *Physiol. Plant* 79:206; Klein et al., (1992), *Biotechnology* 10:268).

Another method for physical delivery of DNA to plants is sonication of target cells as described in Zang et al., (1991), *BioTechnology* 9:996. Alternatively, liposome or spheroplast fusions have been used to introduce expression vectors into plants. See, for example, Deshayes et al., (1985), *EMBO J.* 4:2731; and Christou et al., (1987), *PNAS USA* 84:3962. Direct uptake of DNA into protoplasts using $CaCl_2$ precipitation, polyvinyl alcohol, or poly-L-ornithine has also been reported. See, for example, Hain et al., (1985), *Mol. Gen. Genet.* 199:161; and Draper et al., (1982), *Plant Cell Physiol.* 23:451.

Electroporation of protoplasts and whole cells and tissues has also been described. See, for example, Donn et al., (1990), In: *Abstracts of the VIIth Int'l. Congress on Plant Cell and Tissue Culture IAPTC*, A2-38, page 53; D'Halluin et al., (1992), *Plant Cell* 4:1495-1505; and Spencer et al., (1994), *Plant Mol. Biol.* 24:51-61.

Thus, polynucleotide encoding a polypeptide able to degrade fumonisin or AP1 can be isolated and cloned in an appropriate vector and inserted into an organism normally sensitive to the *Fusarium* or its toxins. Furthermore, the polynucleotide imparting fumonisin or AP1 degradative activity can be transferred into a suitable plasmid, and transformed into a plant. Thus, a fumonisin or AP1 degrading transgenic plant can be produced. Organisms expressing the polynucleotide can be easily identified by their ability to degrade fumonisin or AP1. The protein capable of degrading fumonisin or AP1 can be isolated and characterized using techniques well known in the art.

APAO or trAPAO in a Transgenic Plant

Fumonisin esterase reduces but does not eliminate the toxicity of fumonisins. Therefore a second enzymatic modification to further reduce or abolish toxicity is desirable. The partially purified APAO enzyme from *Exophiala spinifera* has little or no activity on intact FB1, a form of fumonisin. However, recombinant APAO enzyme from *Exophiala spinifera*, expressed in *E. coli*, has significant but reduced activity on intact FB1 and other B-series fumonisins. APAO or trAPAO thus could potentially be used without fumonisin esterase since the amine group is the major target for detoxification. Alternatively, the two genes, fumoninsin esterase and APAO (or trAPAO) can be used together for degrading toxins.

APAO is predicted to be an enzyme that, when by itself or co-expressed in a heterologous expression system along with fumonisin esterase (either ESP1 or BEST1), will result in the production of 2-oxo-FB1 and/or 2-oxo pentol (2-OP) from fumonisin B1. The substrate range of recombinant, *E. coli*-expressed APAO is limited to fumonisins and their hydrolysis products and does not include amino acids, sphingolipid precursors such as phytosphingosine, or polyamines such as spermidine. Thus, APAO is highly specific for fumonisin-like amines, and thus would have little deleterious effect on other cellular metabolites. In addition, if it is extracellularly localized, it will limit any contact with biologically important amines that might also be substrates. The end result will be a more effective detoxification of fumonisins than can be achieved with esterase alone.

The oxidase activity of APAO is predicted to result in generation of hydrogen peroxide in stoichiometric amounts relative to AP1 or fumonisin oxidized. This may prove to be an additional benefit of this enzyme, since hydrogen peroxide is both antimicrobial and is thought to contribute to the onset of a defense response in plants (Przemylaw, *Biochem J.*, 322:681-692 (1997), Lamb, *et al., Ann Rev Plant Physiol Plant Mol Bio* 48:251-275 (1997), and Alverez, *et al., Oxidative Stress and the Molecular Biology of Antioxidant Defenses*, Cold Spring Harbor Press, 815-839 (1997)).

Because one of the embodiments of the present invention is to have both a fumonisin esterase polynucleotide and an APAO or trAPAO polynucleotide present in a plant, there are several ways to introduce more than one polynucleotide in a plant. One way is to transform plant tissue with polynucleotides to both fumonisin esterase and APAO or trAPAO at the same time. In some tissue culture systems it is possible to transform callus with one polynucleotide and then after establishing a stable culture line containing the first polynucleotide, transform the callus a second time with the second polynucleotide. One could also transform plant tissue with one polynucleotide, regenerate whole plants, then transform the second polynucleotide into plant tissue and regenerate whole plants. The final step would then be to cross a plant containing the first polynucleotide with a plant containing the second polynucleotide and select for progeny containing both polynucleotides.

Another method is to create a fusion protein between esterase and APAO or trAPAO, preferably with a spacer region between the two polypeptides. Both enzymes would be

active although tethered to each other. In addition, an enzyme cleavage site engineered in the spacer region, would allow cleavage by an endogenous or introduced protease.

Transgenic plants containing both a fumonisin esterase enzyme and/or the APAO enzyme and thus able to degrade fumonisin or a structurally related mycotoxin would be able to reduce or eliminate the pathogenicity of any microorganism that uses fumonisin or a structurally related mycotoxin as a mode of entry to infect a plant. Fungal pathogens frequently use toxins to damage plants and weaken cell integrity in order to gain entry and expand infection in a plant. By preventing the damage induced by a toxin, a plant would be able to prevent the establishment of the pathogen and thereby become tolerant or resistant to the pathogen.

Another benefit of fumonisin degradation is the production of hydrogen peroxide. When fumonisin or AP1 is oxididatively deaminated at C-2, as occurs by exposure to APAO or trAPAO enzyme, hydrogen peroxide is produced as a by-product. Hydrogen peroxide production can trigger enhanced resistance responses in a number of ways. 1) Hydrogen peroxide has direct antimicrobial activity. 2) Hydrogen peroxide acts as a substrate for peroxidases associated with lignin polymerization and hence cell wall strengthening. 3) Via still to be determined mechanisms, hydrogen peroxide acts as a signal for activation of expression of defense related genes, including those that result in stimulation of salicylic acid accumulation. Salicylic acid is thought to act an endogenous signal molecule that triggers expression of genes coding for several classes of pathogenesis-related proteins. Moreover, salicylic acid may set up the oxidative burst and thus act in a feedback loop enhancing its own synthesis. Salicylic acid may also be involved in hypersensitive cell death by acting as an inhibitor of catalase, an enzyme that removes hydrogen peroxide. 4) Hydrogen peroxide may trigger production of additional defense compounds such as phytoalexins, antimicrobial low molecular weight compounds. For a review on the role of the oxidative burst and SA please see Lamb, C. and Dixon, R.A., *Ann. Rev. Plant Physiol. Plant Mol. Biol.*, 48: 251-275 (1997).

Detoxification of Harvested Grain, Silage, or Contaminated Food Crop

The present invention also relates to a method of detoxifying a fumonisin or a structurally related mycotoxin with an APAO enzyme during the processing of grain for animal or human food consumption, during the processing of plant material for silage, or

food crops contaminated with a toxin producing microbe, such as but not limited to, tomato. Since the atmospheric ammoniation of corn has proven to be an ineffective method of detoxification (see B. Fitch Haumann, *INFORM* 6:248-257 (1995)), such a methodology during processing is particularly critical where transgenic detoxification is not applicable.

In one embodiment of the present invention, fumonisin degradative enzymes are presented to grain, plant material for silage, or a contaminated food crop, or during the processing procedure, at the appropriate stages of the procedure and in amounts effective for detoxification of fumonisins and structurally related mycotoxins. Detoxification by the enzymes, microbial strains, or an engineered microorganism can occur not only during the processing, but also any time prior or during the feeding of the grain or plant material to an animal or incorporation of the grain or food crop into a human food product, or before or during ingestion of the food crop.

Another embodiment of the present invention is the engineering of a bacterium or fungus to express the detoxification enzymes and then using the bacterium or fungus rather than the enzyme itself. There are a number of microbes that could be engineered to express the polynucleotides of the present invention. One could also activate, either inducibly or constitutively, the endogenous genes for fumonisin esterase or APAO. By overexpressing the degradative enzymes and then treating plants, seed, or silage with the microorganism, it would be possible to degrade fumonisin *in situ*.

The polynucleotides of the invention can be introduced into microorganisms that multiply on plants (epiphytes) to deliver enzymes to potential target crops. Epiphytes can be gram-positive or gram-negative bacteria, for example.

The microorganisms that have been genetically altered to contain at least one degradative polynucleotide and resulting polypeptide may be used for protecting agricultural crops and products. In one aspect of the invention, whole, i.e. unlysed, cells of the transformed organism are treated with reagents that prolong the activity of the enzyme produced in the cell when the cell is applied to the environment of a target plant. A secretion leader may be used in combination with the gene of interest such that the resulting enzyme is secreted outside the host cell for presentation to the target plant.

The degradative enzymes can be fermented in a bacterial host and the resulting bacteria processed and used as a microbial spray. Any suitable microorganism can be used

for this purpose. See, for example, Gaertner, *et al.* (1993) in *Advanced Engineered Pesticides,* (ed. Kim, Marcel Dekker, New York).

The enzymes or microorganisms can be introduced during processing in appropriate manners, for example as a wash or spray, or in dried or lyophilized form or powered form, depending upon the nature of the milling process and/or the stage of processing at which the enzymatic treatment is carried out. See generally, Hoseney, R.C., *Principles of Cereal Science and Technology*, American Assn. of Cereal Chemists, Inc., 1990 (especially Chapters 5, 6 and 7); Jones, J.M., *Food Safety*, Eagan Press, St. Paul, MN, 1992 (especially Chapters 7 and 9); and Jelen, P., *Introduction to Food Processing*, Restan Publ. Co., Reston, VA, 1985. Processed grain or silage to be used for animal feed can be treated with an effective amount of the enzymes in the form of an inoculant or probiotic additive, for example, or in any form recognized by those skilled in the art for use in animal feed. The enzymes of the present invention are expected to be particularly useful in detoxification during processing and/or in animal feed prior to its use, since the enzymes display relatively broad ranges of pH activity. The esterase from *Exophiala spinifera*, ATCC 74269, showed a range of activity from about pH 3 to about pH 6, and the esterase from the bacterium of ATCC 55552 showed a range of activity from about pH 6 to about pH 9 (US patent no. 5,716,820, *supra*). The APAO enzyme from *Exophiala spinifera* (ATCC 74269) has a pH range of activity from pH 6 to pH 9.

Genetic Engineering of Ruminant Microorganisms

Ruminant microorganisms can be genetically engineered to contain and express either the fumonisin esterase enzymes or APAO, or a combination of the enzymes. The genetic engineering of microorganisms is now an art recognized technique, and ruminant microorganisms so engineered can be added to feed in any art recognized manner, for example as a probiotic or inoculant. In addition, microorganisms capable of functioning as bioreactors can be engineered so as to be capable of mass producing either the fumonisin esterases or the APAO enzyme.

Use of the Fumonisin Esterase and APAO Enzymes for Detection of Reagents for Fumonisins and Related Compounds

Another embodiment of the present invention is the use of the enzymes of the present invention as detection reagents for fumonisins and related compounds. The enzymes of the present invention can be used as detection reagents because of the high specificity of the esterase and deaminase enzymes, and the fact that hydrolysis followed by amine oxidation can be monitored by detection of hydrogen peroxide or ammonia using standard reagents (analogous to a glucose detection assay using glucose oxidase). Hydrogen peroxide is often measured by linking a hydrogen peroxide-dependent peroxidase reaction to a colored or otherwise detectable peroxidase product (e.g. Demmano, *et al., European Journal of Biochemistry* 238(3): 785-789 (1996)). Ammonia can be measured using ion-specific electrodes : Fritsche, *et al., Analytica Chimica Acta* 244(2): 179-182 (1991); West, *et al., Analytical Chemistry* 64(5): 533-540 (1992), and all herein incorporated by reference) or by GC or other chromatographic method.

For example, recombinant or non-recombinant, active fumonisin esterase (ESP1 or BEST) and APAO proteins are added in catalytic amounts to a sample tube containing an unknown amount of fumonisins (FB1, FB2, FB3, FB4, or partial or complete hydrolysis products of these). The tube is incubated under pH and temperature conditions sufficient to convert any fumonisin in the sample to AP1 or to 2-oxo-FB1, and correspondingly the AP1 to 2-OP, ammonia, and hydrogen peroxide. Alternatively, APAO or trAPAO is added in catalytic amounts to a sample tube containing an unknown amount of fumonisins (FB1, FB2, FB3, FB4, or partial or complete hydrolysis products of these). The tube is incubated under pH and temperature conditions sufficient to convert any fumonisin in the sample to 2-oxo FB1, ammonia, and hydrogen peroxide. Then suitable reagents are added for quantification of the hydrogen peroxide or ammonia that were generated stoichiometrically from fumonisins. By comparison with control tubes that received no esterase or APAO enzyme, the amount of fumonisin present can be calculated in direct molar proportion to the hydrogen peroxide or ammonia detected, relative to a standard curve.

This invention can be better understood by reference to the following non-limiting examples. It will be appreciated by those skilled in the art that other embodiments of the invention may be practiced without departing from the spirit and the scope of the invention as herein disclosed and claimed.

Example 1

Fungal and bacterial isolates. *Exophiala* isolates from maize were isolated as described in US patent no. 5,716,820, issued February 10, 1998; US patent no. 6,025,188, issued February 15, 2000; and pending US application no. 08/888,950, filed July 7, 1997, and herein incorporated by reference.

Isolation methods. Direct isolation of black yeasts from seed was accomplished by plating 100 microliters of seed wash fluid onto YPD or Sabouraud agar augmented with cycloheximide (500 mg/liter) and chloramphenicol (50 mg/liter). Plates were incubated at room temperature for 7-14 days, and individual pigmented colonies that arose were counted and cultured for analysis of fumonisin-degrading ability as described in US patent no. 5,716,820, issued February 10, 1998; US patent no. 6,025,188, issued February 15, 2000; and pending US application no. 08/888,950, filed July 7, 1997.

Analysis of fumonisins and metabolism products. Analytical thin-layer chromatography was carried out on 100% silanized C18 silica plates (Sigma #T-7020; 10 x 10 cm; 0.1 mm thick) by a modification of the published method of Rottinghaus (Rottinghaus, *et al.*, *J Vet Diagn Invest,* 4: 326 (1992), and herein incorporated by reference).

To analyze fumonisin esterase activity sample lanes were pre-wet with methanol to facilitate sample application. After application of from 0.1 to 2 μl of aqueous sample, the plates were air-dried and developed in MeOH:4% KCl (3:2) or MeOH:0.2 M KOH (3:2) and then sprayed successively with 0.1 M sodium borate (pH 9.5) and fluorescamine (0.4 mg/ml in acetonitrile). Plates were air-dried and viewed under long wave UV.

For analysis of APAO activity, an alternative method was used. Equal volumes of sample and ^{14}C-AP1 (1 mg/ml, pH 8, 50 mM sodium phosphate) were incubated at room temperature for one to six days. Analytical thin-layer chromatography was then carried out on C60 HPK silica gel plates (Whatman #4807-700; 10x10 cm; 0.2 mm thick). After application of from 0.1 to 2 μl of aqueous sample, the plates were air-dried and developed in $CHCl_3$:MeOH:CH_3COOH:H_2O (55:36:8:1). Plates were then air dried, and exposed to PhosphorImager screen (Molecular Dynamics) or autoradiographic film. A Storm™ PhosphorImager (Molecular Dynamics) was used to scan the image produced on the screen.

Alkaline hydrolysis of FB1 to AP1. FB1 or crude fumonisin C_8 material was suspended in water at 10-100 mg/ml and added to an equal volume of 4 N NaOH in a screw-cap tube. The tube was sealed and incubated at 60°C for 1 hr. The hydrolysate was cooled to RT and mixed with an equal volume of ethyl acetate, centrifuged at 1000 RCF for 5 minute and the organic (upper) layer recovered. The pooled ethyl acetate layers from two successive extractions were dried under N_2 and resuspended in distilled H_2O. The resulting material (the aminopentol of FB1 or "AP1") was analyzed by TLC.

Enzyme activity of culture filtrate and mycelium. *Exophiala spinifera* isolate 2141.10 was grown on YPD agar for 1 week, and conidia were harvested, suspended in sterile water, and used at 10^5 conidia per ml to inoculate sterile Fries mineral salts medium containing 1 mg/ml purified FB1 (Sigma Chemical Co.). After 2 weeks incubation at 28° C in the dark, cultures were filtered through 0.45 micron cellulose acetate filters, and rinsed with Fries mineral salts. Fungal mycelium was suspended in 15 mL of 0.1% FB1, pH 5.2 + 1 mM EDTA + 3 μg/mL Pepstatin A + 1.5 μg/mL Leupeptin and disrupted in a Bead Beater™ using 0.1 mm beads and one minute pulses, with ice cooling. Hyphal pieces were collected by filtering through Spin X™ (0.22 μm), and both mycelial supernatant and original culture filtrates were assayed for fumonisin modification by methods outlined above.

Preparation of crude culture filtrate. Agar cultures grown as above were used to inoculate YPD broth cultures (500 ml) in conical flasks at a final concentration of 10^5 conidia per ml culture. Cultures were incubated 5 days at 28°C without agitation and mycelia harvested by filtration through 0.45 micron filters under vacuum. The filtrate was discarded and the mycelial mat was washed and resuspended in sterile carbon-free, low mineral salts medium (1 g/liter NH_3NO_4; 1 g/liter NaH_2PO_4; 0.5 g/liter $MgCl_2$; 0.1 g/liter NaCl; 0.13 g/liter $CaCl_2$; 0.02 g/liter $FeSO_4 \cdot 7H_20$, pH 4.5) containing 0.5 mg/ml alkaline hydrolyzed crude FB1. After 3-5 days at 28°C in the dark with no agitation the cultures were filtered through low protein binding 0.45 micron filters to recover the culture filtrate. Phenylmethyl sulfonyl fluoride (PMSF) was added to a concentration of 2.5 mM and the culture filtrate was concentrated using an Amicon™ YM10 membrane in a stirred cell at room temperature, and resuspended in 50 mM sodium acetate, pH 5.2 containing 10 mM $CaCl_2$. The crude culture filtrate (approx. 200-fold concentrated) was stored at -20°C.

To obtain preparative amounts of enzyme-hydrolyzed fumonisin, 10 mg. of FB1 (Sigma) was dissolved in 20 mL of 50 mM sodium acetate at pH 5.2 + 10 mM $CaCl_2$, and 0.25 mL of 200x concentrated crude culture filtrate of 2141.10 was added. The solution was incubated at 37°C for 14 hours, and then cooled to room temperature. The reaction mixture was brought to approx. pH 9.5 by addition of 0.4 mL of 4 N KOH, and the mixture was extracted twice with 10 mL ethyl acetate. The combined organic layers were dried under N_2 and resuspended in dH_2O. 2.5 milligrams of organic extracted material were analyzed by Fast Atom Bombardment (FAB) mass spectrometry. The resulting mass spectrum showed a major ion at M/z (+1)=406 mass units, indicating the major product of enzymatic hydrolysis was AP1, which has a calculated molecular weight of 405.

Example 2

Preparation of AP1-induced and non-induced mycelium.

Liquid cultures of *Exophiala spinifera* isolate 2141.10 were prepared from YPD agar plates (Yeast Extract 10 gm, Bacto-Peptone 20 gm, Dextrose 0.5 gm, and Bacto-Agar 15 gm per liter of water). Aliquots (400-500 uL) of a water suspension of *E. spinifera* cells from YPD agar were spread uniformly onto 150 x 15 mm YPD agar plates with 4 mm sterile glass beads. The plates were incubated at room temperature for 6-7 days. The mycelia/conidia were transferred from the agar plates into Mineral Salts Medium (MSM) ($Na_2HPO_4{\cdot}7H_2O$ 0.2 gm, NH_4Cl 1.0 gm, $CaCl_2{\cdot}2H_2O$ 0.01 gm, $FeSO_4{\cdot}7H_2O$ 0.02 gm per liter of distilled water, pH 4.5) and centrifuged at 5000 x g, 4°C, 20 minutes to pellet the cells. The cell pellet was rinsed once in 40 ml MSM and recentrifuged. The rinsed cell pellet was used to inoculate MSM at a 1:19 ratio of packed cells: MSM. The culture to be induced was supplemented with AP1 to a final concentration of 0.5-1.0 mg/ml and incubated at 28 °C, 100 rpm, in the dark to induce catabolic enzymes. The non-induced cultures did not receive AP1 but were grown on media containing 4-ABA at the same concentration as AP1. The supernatants were removed by filtration through 0.45 cellulose acetate. The remaining mycelial mat was washed with sterile MSM and then frozen in liquid nitrogen for storage.

Example 3

Effect of FB1 and AP1 on maize coleoptiles

Maize coleoptiles from 4 day dark-grown germinated maize seeds were excised above the growing point and placed in 96-well microtiter plates in the presence of 60 microliters of sterile distilled water containing FB1 or AP1 at approximately equimolar concentrations of 1.5, .5, .15, .05, .015, .005, .0015, or .0005 millimolar, along with water controls. After 2 days in the dark at 28° C the coleoptiles were placed in the light and incubated another 3 days. Injury or lack thereof was evaluated as follows:

	0	.0005	.0015	.005	.015	.05	.15	.5	1.5	mM
FB1	-	-	-	-	+/-	+	+	+	+	
AP1	-	-	-	-	-	-	-	-	+	

+ = brown necrotic discoloration of coleoptile

- = no symptoms (same as water control)

The results (see table above) indicate there is at least a 30-fold difference in toxicity between FB1 and AP1 to maize coleoptiles of this genotype. This is in general agreement with other studies where the toxicity of the two compounds was compared for plant tissues: In *Lemna* tissues, AP1 was approx. 40-fold less toxic (Vesonder *et al.,*" *Arch Environ Contam Toxicol* 23: 464-467 (1992).). Studies with both AAL toxin and FB1 in tomato also indicate the hydrolyzed version of the molecule is much less toxic (Gilchrist *et al., Mycopathologia* 117: 57-64 (1992)). Lamprecht *et al.* also observed an approximate 100-fold reduction in toxicity to tomato by AP1 versus FB1 (Lamprecht *et al., Phytopathology* 84: 383391 (1994))

Example 4

Effect of FB1 and AP1 on maize tissue cultured cells (Black Mexican Sweet, BMS)

FB1 or AP1 at various concentrations was added to suspensions of BMS cells growing in liquid culture medium in 96-well polystyrene plates. After 1 week the cell density in wells was observed under low power magnification and growth of toxin-treated wells was compared to control wells that received water. Growth of BMS cells was significantly inhibited at 0.4 micromolar FB1, but no inhibition was observed until 40 micromolar AP1. This represents an approximate 100-fold difference in toxicity to maize tissue cultured cells. Similarly Van Asch et al. (VanAsch *et al., Phytopathology* 82: 1330-

1332 (1992)) observed significant inhibition of maize callus grown on solid medium at 1.4 micromolar FB1. AP1 was not tested in that study, however.

Example 5

APAO Activity

A cell-free extract that contains the deaminase activity was obtained by subjecting substrate-induced *Exophiala spinifera* cells to disruption using a Bead Beater™ in 50 mM Na-phosphate, pH 8.0, and recovering the cell-free supernatant by centrifugation and .45 micron filtration. Catabolic activity is assayed by incubating extracts with AP1 (hydrolyzed fumonisin B1 backbone) or ^{14}C-labelled AP1 with the extract and evaluating by TLC on C18 or C60 silica. The product 2-OP has a lower Rf than AP1 and is detected either by radiolabel scan or by H_2S0_4 spray/charring of the TLC plate. 2-OP does not react with the amine reagent, fluorescamine, that is routinely used to detect AP1 on TLC plates, suggesting that the amine group is missing or chemically modified. Activity is greater at 37°C than at room temperature, but following 30 min. at 65°C or 100°C (no AP1 catabolic activity remained). Activity is maximal at pH 9. At pH 9, complete conversion to 2-OP occurred in 30 minutes. Activity is retained by 30,000 dalton molecular weight cutoff membrane, but only partially retained by 100,000 dalton molecular weight cutoff membrane. Other amine-containing substrates were tested for modification by the crude extract. Fumonisin, with tricarballylic acids attached, is not modified by the extract, indicating that ester-hydrolysis must occur first for the APAO to be able to be effective in modifying FB1 (as noted below, the *E. coli*-expressed, recombinant APAO enzyme does in fact oxidize FB1 although at a lower rate than AP1). Other long-chain bases (sphingosine, sphinganine, and phytosphingosine) are apparently not modified by the crude APAO, suggesting the enzyme(s) is specific for the fumonisin backbone. Preparative amounts of the product, named 2-OP, have also been purified and analyzed by C13 nmr. The results indicate that 2-OP has a keto group at carbon 2 instead of an amine, consistent with an oxidative deamination by an amine oxidase. The C13 nmr data also indicate that 2-OP spontaneously forms an internal hemiketal between C-1 and C-5, resulting in a 5-membered ring with a new chiral center at C-2. All other carbon assignments are as in AP1, thus 2-OP is a compound of composition $C_{22}H_{44}O_6$, FW 404. The product of the

enzyme acting on hydrolyzed fumonisin would not be expected to display any significant toxicity.

Other enzymes were tested for their ability to modify AP1. All enzymes were assayed by radiolabeled TLC, as described above, under optimal conditions at 37^0 Celsius, overnight or longer. The results are as follows:

Deaminating	**EC**	**Source**	**Result**
Monoamine Oxidase	1.4.3.4	bovine plasma	negative
D-amino oxidase	1.4.3.3	porcine kidney; TypeX	negative
L-amino oxidase	1.4.3.2	C.adamanteus venom; TypeI	negative
Tyramine oxidase	1.4.3.4	Arthrobacter spp	negative
Methylamine dehydrogenase	1.4.99.3	Paracoccus denitrificans	negative
Aralkyl amine dehydrogenase	1.4.99.4	Alcaligenes faecalis	negative
Phenylalanine ammonia lyase	4.3.1.5	Rhodotorula glutinis; TypeI	negative
Histidine ammonia lyase	4.3.1.3	Pseudomonas fluorescens	negative
L-aspartase	4.3.1.1	Hafnia alvei (Bacterium cadaveris)	negative
Tyrosine oxidase	1.14.18.1	mushroom	negative
Lysine oxidase	1.4.3.14	Trichoderma viride	negative
Diamine oxidase	1.4.3.6	porcine kidney	negative

The results were negative for each enzyme tested. Therefore isolates from the American Type Culture Collection (ATCC) were collected. The ATCC isolates selected were listed as containing amine-modifying enzymes or were capable of growth/utilization on amine-containing substrates. The isolates were tested to determine if they could grow on or utilize AP1 as the sole carbon source and if any could modify AP1 to a new compound(s). The nitrogen sources that were used in liquid cultures were AP1 0.1% (w/v), s-butylamine 0.1% (v/v), n-butylamine 0.1% (v/v), and ammonium nitrate 0.2% (w/v). These were prepared in Vogel's Minimal Media (without NH_4NO_3) containing 2% sucrose. The isolates were inoculated into the various media and monitored for growth over 2-3 weeks. They were also assayed with the ^{14}C-radiolabeled TLC assay for AP1 modification. In summary, none of the isolates tested exhibited modification of AP1 *in vivo.* Clearly the APAO enzyme is unique and unusual in its ability to modify the AP1 toxin.

EXAMPLE 6

Isolation of the trAPAO Polynucleotide

The trAPAO polynucleotide was identified using a proprietary transcript imaging method that compares transcript patterns in two samples and allows cloning of differentially expressed fragments. This technology was developed by CuraGen® (New

Haven, CT). (see Published PCT patent application no. WO 97/15690, published May 1, 1997, and hereby incorporated by reference) Fluorescently-tagged, PCR amplified cDNA fragments representing expressed transcripts can be visualized as bands or peaks on a gel tracing, and the cDNA from differentially expressed (induced or suppressed) bands can be recovered from a duplicate gel, cloned and sequenced. Known cDNAs can be identified without the need for cloning, by matching the predicted size and partially known sequence of specific bands on the tracing.

In the present invention two RNA samples were obtained from cultures of *E. spinifera* grown for a specified period in a mineral salts medium containing either AP1 (induced condition), or gamma-aminobutyric acid (ABA; non-induced condition) as a sole carbon source. In the induced condition, fumonisin esterase and APAO enzyme activities are detected, whereas in the non-induced condition these activities are not detected. The methods used for induction of APAO and detection of activity are described earlier (see Example 2 and Example 5). RNA was extracted from induced mycelium by Tri-Reagent methods (Molecular Research Center Inc., Cincinnati, Ohio) only grinding a frozen slurry of tissue and Tri-Reagent with a mortar and pestle until almost melted and adding an additional extraction after the phase separation by extracting the aqueous phase one time with phenol, and two times with a phenol:chloroform:isoamyl alcohol mixture. The RNA's were submitted for CuraGen® transcript imaging to detect cDNA fragments that are induced specifically in the presence AP1. In the resulting gel tracing several bands were found which showed induction of at least 2-fold and up to 79-fold or even 100-fold or more in AP1. In the resulting gel tracing several bands were found which showed induction of at least 10-fold in AP1-grown cells as compared to cells grown in ABA. The sequence of two highly induced bands can be found in Table 1.

TABLE 1

Nucleotide sequence of two CuraGen® bands that were identified as strongly induced by AP1 in cultures of *Exophiala spinifera.*

>k0n0-395.5_b (SEQ ID NO: 1)
GGGCCCCGGCGTTCTCGTAGGCTGCGCGGAGTTGGTCCCAGACAGACTTTTGTCGTACCTGCTTG
GACTGTTGGGACCACTTCCGTCCCGGGTCTCCGACCATGAAACAGGTAATGGACCATTGTCGAT
CGACGTCGATGCTGGTATCTCTGGCAAATGAGATGGGGTCACAGCTCGATTGGAGGACGCCCGA
GAAGCCTTGTTCGCGCCACCACGGCTTGTCCCATACGAAGACTATCTTGCTATAGTAGCCCAGG
ATAGAATTTTCCGCCAATGCTTGCTTCTCGGCGGGAAGAGGTGGTGAAAATGTCAAGGTGGGAT
ACAAGGTTGTCGGTAACGAAACCANCACCTTTTTGCTTCGGAACACGGCGC

>r0c0-182.3_6 (SEQ ID NO: 2)
GAATTTTCCGCCAATGCTTGCTTCTCGGCGGGAAGAGGTGGTGAAAATGTCAAGGTGGGATACA
AGGTTGTCGGTAACGAAACCACCACCTTTTTGCTTCGGAACACGGCGCCCGAGGCCGATCGTAC
TGTACAGCCGGATGCCGACTGCTCAATTTCAGCGACGGGGGTGTTGAGGTGCAC

Two of the highly induced bands, k0n0-395.5, and r0c0-182.3 showed significant sequence homology to a family of enzymes, flavin-containing amine oxidases (EC 1.4.3.4), that oxidize primary amines to an aldehyde or ketone, releasing ammonia and hydrogen peroxide (Table 2).

TABLE 2

Identification of a putative flavin amine oxidase from *E. spinifera:* AP1-induced transcript fragments with amine oxidase homology. BLAST 2.0 default parameters.

Clone ID	Size	Best Hit	Best Hit Name, source	Prob	from	to	Likely function
k0n0-395.5	395 bp	P40974	putrescine oxidase, *Micrococcus rubens,* EC 1.4.3.10 Length = 478	8.0 e -07	276	333	oxidation of C-2 amine of AP1
r0c0-182.3 (contigs with k0n0-395)	182 bp	P12398	monoamine oxidase type A (MAO-A) [*Bos taurus*] Length = 527	0.0039	238	296	oxidation of C-2 amine of AP1

The chemical structure of the primary product of AP1 deamination is thought to be a 2-keto compound which cyclizes to a hemiketal at carbons 2 and 5. Therefore it is predicted that this induced enzyme is responsible for deamination of AP1.

Using sequence derived from k0n0-395.5, a partial cDNA was obtained by 3' and 5' RACE-PCR (Chenchik, *et al., CLONTECHniques* X 1:5-8 (1995); Chenchik, *et al.,* A new method for full-length cDNA cloning by PCR. In *A Laboratory Guide to RNA: Isolation, Analysis, and Synthesis*. Ed. Krieg, P.A. (Wiley-Liss, Inc.), 273-321 (1996)). A RACE cloning kit from CLONTECH was used, to obtain the RACE amplicons. Briefly, poly A+ RNA is transcribed to make first strand cDNA using a "lock-docking" poly T, cDNA synthesis primer, the second strand is synthesized and the

Marathon cDNA adaptor is ligated to both ends of the ds cDNA. Diluted template is then used with the Marathon adapter primer and in separate reactions either a 5' Gene Specific Primer (GSP) or a 3'GSP is used to produce the 3' or 5' RACE amplicon. After characterization of the RACE product(s) and sequencing, full-length cDNAs may be generated by 1) end-to-end PCR using distal 5' and 3' GSPs with the adapter-ligated ds cDNA as template, or 2) the cloned 5' and 3'-RACE fragments may be digested with a restriction enzyme that cuts uniquely in the region of overlap, the fragments isolated and ligated. Subsequently, the RACE-generated full-length cDNAs from 1) and 2) may be cloned into a suitable vector.

In combination with the supplied adapter primer the following gene specific primers were used: for 3' RACE the oligonucleotide N21965: 5'-TGGTTTCGTTACCGACAACCTTGTATCCC-3' (SEQ ID NO: 3) and for 5' race, the oligonucleotide N21968: 5'-GAGTTGGTCCCAGACAGACTTTTGTCGT-3' (SEQ ID NO: 4. The polynucleotide sequence of the trAPAO polynucleotide, k0n0-395_6.5, from *Exophiala spinifera* is shown in SEQ ID NO: 5. The polypeptide sequence of trAPAO is shown in SEQ ID NO: 6.

A second clone of APAO containing an unspliced intron was also found. The polynucleotide sequence of trAPAO-I polynucleotide, k0n0-395_5.4, the intron containing clone, from *Exophiala spinifera*, can be found in SEQ ID NO: 7. The polypeptide sequence of trAPAO-I with the intron spliced out is shown in SEQ ID NO: 8. The polypeptide sequence of trAPAO-I without the intron spliced out is shown in SEQ ID NO: 9.

EXAMPLE 7

Heterologous Expression of trAPAO

Protein alignments generated with PileUp (GCG) indicate that k0n0-395_6.5 (trAPAO) is similar in size to other flavin amine oxidases and is close to being full length with respect to the amino terminus of their class of proteins. The k0n0-395_6.5 sequence contains a complete $\beta-\alpha-\beta$ fold that is required for dinucleotide (FAD) binding, close to the amino end. The k0n0-395 sequence appears to lack only a variable amino terminal segment that varies in length from 5 amino acids in rat monoamine oxidases A & B to 40 amino acids in length in *Aspergillus* MAO-N. The function of these amino terminal extensions is not known; they are not recognizable as secretion signals. Based on the likely

localization of the *Exophiala* APAO outside the cell membrane, the prediction is that k0n0-395 would have a signal sequence similar to that of the fumonisin esterase cloned from the same organism (US patent no. 5,716,820, *supra*). Using GenomeWalker ™, it is possible to clone the 5' end of the transcript and upstream genomic regulatory elements. However, the signal sequence is not expected to be critical to the functionality of the enzyme; in fact, the preferred strategy for heterologous expression in maize and *Pichia pastoris* involves replacing the endogenous signal sequence (if present) with an optimized signal sequence for the organism, e.g. barley alpha amylase for maize and the yeast alpha factor secretion signal for *Pichia*. In maize transformed with fumonisin esterase, the barley alpha amylase signal sequence gave higher amounts of functional protein than the native fungal signal, therefore replacement of the native fungal signal sequence is a logical optimization step. Since many of the amine oxidases have a positively charged amino acid near the N-terminus and upstream of the dinucleotide binding site, an additional optimization step included adding a codon for the lysine (K) to the N-terminus of the trAPAO clone (k0n0-395_6.5, SEQ ID NO: 5). This clone is designated K:trAPAO and can be seen in SEQ ID NOS: 10 and 11. The extra lysine is at amino acid 1 and nucleotides 1-3.

EXAMPLE 8

Pichia Expression of trAPAO

For optimum expression of trAPAO in *Pichia pastoris* the alpha mating factor signal peptide was operably linked in-frame with K:trAPAO coding sequence and can be seen in SEQ ID NOS: 16 and 17. The nucleotide sequence of clone pPicZalphaA:K:trAPAO contains a PCR-amplified insert comprising the k0n0-395 open reading frame with an additional lysine residue at the amino terminus, with a 5' EcoRI site and 3' NotI site for in-frame cloning into the alpha factor secretion vector pPicZalphaA. Nucleotides 1-267 contain the yeast α mating factor secretion signal. The amino acid sequence, shown in SEQ ID NO: 17, contains the trAPAO polypeptide produced from pPicZalphaA:K:trAPAO following transformation into *Pichia pastoris*.

For cloning into expression vectors, two cloning strategies were used. The cDNA k0n0-395_5.4 was generated by using end-to-end PCR using distal 5' and 3' GSPs with the adapter-ligated double stranded cDNA as a template. Each oligonucleotide primer was

designed with 5' restriction enzyme sites that contain a 23-25 bp of anchored gene sequence. The 3' primer also included the stop codon. The primer sequences are N23256: 5'-ggggaattcAAAGACAACGTTGCGGACGTGGTAG-3' (SEQ ID NO: 12) and N23259: 5'-ggggcggccgcCTATGCTGCTGGCACCAGGCTAG-3' (SEQ ID NO: 13). A second method was used to generate k0n0-395_6.5. 5' RACE and 3' RACE products using a distal primer containing the necessary restriction enzyme sites, stop codon, etc as described above and paired with a "medial" GSP. The "medial primers" N21965: 5'-TGGTTTCGTTACCGACAACCTTGTATCCC-3' (SEQ ID NO: 14) for 3' RACE and for 5' race, the oligonucleotide N21968: 5'-GAGTTGGTCCCAGACAGACTTTTGTCGT-3' (SEQ ID NO: 15). Adapter-ligated double stranded cDNA was used as template. The isolated 5' and 3'-RACE fragments were digested with a restriction enzyme that cuts uniquely in the region of overlap, in this case Bgl I, isolated and ligated into the expression vector. The digestible restriction sites allow cloning of the inserts in-frame into EcoRI/NotI digested pPicZalphaA. pPicZalphaA is an *E. coli* compatible *Pichia* expression vector containing a functional yeast alpha factor secretion signal and peptide processing sites, allowing high efficiency, inducible secretion into the culture medium of *Pichia*. The resulting 1.4 kb bands were cloned into EcoRI/NotI digested pPicZalphaA plasmid.

SEQ ID NO: 16 contains the polynucleotide sequence of clone pPicZalphaA:K:trAPAO, a PCR-amplified insert that comprises the k0n0-395 open reading frame with an additional lysine residue at the amino terminus, and a 5' EcoRI site and 3' NotI site for in-frame cloning into the alpha factor secretion vector pPicZalphaA. SEQ ID NO: 17 contains the amino acid sequence of the trAPAO polypeptide produced from pPicZalphaA:K:trAPAO following transformation into *Pichia pastoris*. The alpha factor secretion signal and a lysine are added.

Pichia was transformed as described in Invitrogen Manual, Easy Select™ Pichia Expression Kit, Version B, #161219, with the trAPAO polynucleotide as described above with either an intron (trAPAO-I, negative control, no expression of active trAPAO since *Pichia* does not splice introns very efficiently) or without an intron (capable of making an active APAO protein). The *Pichia* culture fluids and pellets were assayed for APAO activity as described earlier.

The set of frozen six day *Pichia* culture cell pellets contained two samples with intron (SEQ ID NO: 7) in gene construct, # 11, # 14, and two samples without intron in

gene construct (SEQ ID NO: 5), #6, # 52. The six day culture fluids from the same cultures were used to spike with crude fungal enzyme for positive controls.

The 50 μl cell pellets were resuspended in 150 μl cold 50mM Na-phosphate, pH 8.0, and divided into two fresh 500 μl tubes. One tube was kept on ice with no treatment, the pellet suspension, and one tube was used for lysis. An equal volume of 0.1 mm zirconia-silica beads was added to each tube. The tubes were BeadBeat™ for 15 seconds then cooled on ice 5 minutes. This was repeated three times. The crude lysate was then transferred to another tube for assay or lysate suspension.

The TLC assays were performed as follows, the samples are 1) pellet suspensions; 10 μl; 2) lysate suspensions; 10 μl; 3) media controls-mixed 5 μl media with 5 μl crude fungal enzyme; 10 μl; 4) positive control-used crude fungal enzyme undiluted; 10 μl; 5) substrate control-used 50mM Na-phosphate, pH8.0; 10 μl. Ten microliters of each sample plus 10 μl of ^{14}C-AP1 (1 mg/ml, 50 mM Na-phosphate, pH 8) was incubated at room temperature for 6 days. One microliter of the sample was spotted onto C18 and C60 TLC plates. The C18 plates were developed in MeOH:4% KCl (3:2). The C60 plates were developed in $CHCl_3$:MeOH:CH_3COOH:H_2O (55:36:8:1). The plates were then air dried and then exposed to a PhosphorScreen™ for 2-3 days. A Storm™ PhosphorImager was used to develop the images.

A positive TLC result is obtained if an additional radioactive spot appears at a lower Rf of the produced AP1 modification earlier identified as 2-OP, a deaminated product of AP1. In samples # 6 and # 52 (without intron) the AP1-modifying enzyme activity (conversion of AP1 to 2-OP) was detected in pellet suspensions and pellet lysates, although the majority of activity was associated with the pellet suspensions. In samples #11 and #14 (with intron) a minimal amount of AP1-modifying enzyme activity was detectable in the pellet lysate of # 14 only, which indicates Pichia cannot process the intron efficiently.

This experiment verified APAO activity can be detected in *Pichia* transformants, which verifies that trAPAO as described functions correctly in degrading AP1. The activity is associated with cell suspensions, which show higher activity than pellet lysates. Pellet lysates may show less activity due to release of endogenous proteases during lysis of the cells.

EXAMPLE 9

Expression of trAPAO or APAO in *E. coli*

The vector for expressing K:trAPAO in *E. coli* is pGEX-4T-1. This vector is a prokaryotic glutathione S-transferase (GST) fusion vector for inducible, high-level intracellular expression of genes or gene fragments as fusions with *Schistosoma japonicum* GST. GST gene fusion vectors include the following features, a lac promoter for inducible, high-level expression; an internal lac Iq gene for use in any *E. coli* host; and the thrombin factor Xa or PreScission Protease recognition sites for cleaving the desired protein from the fusion product. The insert of interest, k0n0-395_6.5 (K:trAPAO) or APAO, was subcloned into the 5' EcoRI site and a 3' NotI site allowing in-frame expression of the GST:K:trAPAO or GST:APAO fusion peptide.

The polynucleotide sequence of the GST:K:trAPAO fusion can be found in SEQ ID NO: 18. The GST fusion with polylinker can be found at nucleotides 1 to 687. The K:trAPAO can be found at nucleotides 688 to 2076. The resulting polypeptide for the GST:K:trAPAO fusion can be seen at SEQ ID NO: 19. Amino acids 1 to 229 represent the GST fusion plus polylinker and amino acids 230 to 692 represent the K:trAPAO portion of the fusion.

E. coli was transformed with the pGEX-4T-1 vector containing K:trAPAO or APAO as described in BRL catalogue, Life Technologies, Inc. catalogue; Hanahan, D., *J. Mol. Biol.* 166:557 (1983) Jessee, J. *Focus* 6:4 (1984); King, P.V. and Blakesley, R., Focus 8:1, 1 (1986), and hereby incorporated by reference. The transformed *E. coli* was induced by addition of IPTG (isopropyl b-D-thiogalactopyranoside). Four samples of soluble extract and four samples of insoluble inclusion bodies were tested for trAPAO or APAO activity as described in Example 8. APAO activity was present in all soluble samples and two insoluble samples. Highest activity was found at 10 uM IPTG induction. Thus the pGEX-4T-1 vector containing K:trAPAO or APAO is capable of producing active APAO enzyme in *E. coli*.

EXAMPLE 10

The Complete Nucleotide Sequence of the *Exophiala* APAO Gene

Using Genome Walker, the complete nucleotide sequence of the *Exophiala* APAO gene was recovered. The nucleotide sequence described in SEQ ID NO: 5 is missing a

portion of the 5' end of the native gene. The missing portion of the 5' end of the native gene is not necessary for expression of an active APAO enzyme, as can be seen in Examples 8 and 9. The complete nucleotide sequence of APAO can be seen in SEQ ID NO: 22. The translation of SEQ ID NO: 22 can be found in SEQ ID NO: 23.

EXAMPLE 11

Expression of APAO and ESP1 in transgenic maize callus

One of the preferred constructs for expression in maize is the nucleotide sequence of the trAPAO operably linked to the barley alpha amylase signal sequence. The nucleotide sequence of K:trAPAO translational fusion with barley alpha amylase signal sequence, for expression and secretion of the mature trAPAO in maize can be seen in SEQ ID NO: 20. Nucleotides 1-72, represent the barley alpha amylase signal sequence; nucleotides 73-75, represent the added lysine residue; and nucleotides 76 -1464 , represent the trAPAO cDNA. The amino acid sequence translation of SEQ ID NO: 20 can be found in SEQ ID NO: 21. Amino acids 1 to 24 represent the barley alpha amylase signal sequence and amino acids 25 to 463 is the sequence of K:trAPAO.

Maize embryos were transformed with linear DNA (insert, lacking a bacterial antibiotic resistance marker), derived from constructs containing three transcription units: 1) a PAT selectable marker gene (Wohlleben *et al.*, *Gene* 70, 25-37 (1988)), 2) fumonisin esterase ESP1 operably linked to a barley alpha amylase signal sequence, and 3) full length APAO without or with an amino-terminal barley alpha amylase signal sequence, (P13603, comprising a PAT selectable marker operably linked to a 35S promoter, fumonisin esterase ESP1 operably linked to a barley alpha amylase signal sequence and the ubiquitin promoter, and APAO operably linked to the ubiquitin promoter and P13611, comprising a PAT selectable marker operably linked to the 35S promoter, fumonisin esterase ESP1 operably linked to a barley alpha amylase signal sequence and the ubiquitin promoter and APAO operably linked to a barley alpha amylase signal sequence and the ubiquitin promoter). In these constructs both ESP1 and APAO were linked to the maize ubiquitin promoter and first intron. In a third construct, the same three transcriptional units were cloned into an Agrobacterium T1 vector (P15258, the construct comprises a PAT selectable marker, fumonisin esterase ESP1 operably linked to a barley alpha amylase signal sequence and APAO). Stably transformed callus or T0 plants regenerated from callus were tested for ESP1 and APAO activity in buffer extracts of leaf tissue, using

radiolabeled FB1 and/or AP1 and C18 thin-layer chromatography. Positive controls consist of non-transformed tissue spiked with *E coli*-expressed recombinant ESP1 or APAO. The results indicate that both ESP1 and APAO activities can be detected in transgenic maize callus and plants.

Expression of ESP1 and APAO in transgenic callus

Construct	Sample ID Number	ESP1 activity (TLC)	APAO activity (TLC)
13603	3065.031-2	+	+
13603	3065.034-3	+	+
13603	3065.1117-3	+	+
13603	3065.11s7-n13	+	+
13603	3065.117-2	+	+
13603	3065.1115-2	+	+
13603	3065.1115-6	+	+
13603	3065.1112-1	+	+
13603	3065.118-6	+	+
13603	3065.11s3-1	+	+
13603	3065.11s1-13	+	+
13603	2805.762-2	+	+
13603	3065.1110-2	+	+
13603	3065.039-2	+	+
13611	3065.293-3	+	+
13611	3065.263-1	+	+
13611	3070.24.2.3	+	+

Transgenic plants were regenerated from the transgenic callus positive for both ESP1 and APAO activity by standard methods known in the art. Enzyme activity was tested as described previously. As can be seen below transgenic maize plants can successfully express both ESP1 and APAO enzymes.

Expression of APAO and ESP1 in transgenic maize plants (T0)

Construct	Sample ID Number	ESP1 activity (TLC)	APAO activity (TLC)
13603	910080	+	+
13603	910081	+	+
13603	917065	+	+

Another preferred construct for expression of APAO in a plant is targeting the APAO to the peroxisome. Maize embryos were bombarded with insert containing APAO

operably linked to ubiquitin promoter and a peroxisomal targeting sequence (Gould, *et al.*, *J Cell Biol* 108:1657-1664 (1989)); ESP1 operably linked to ubiquitin promoter and the barley alpha amylase signal sequence; and a selectable marker of PAT operably linked to the 35S promoter (construct number I14952). Negative controls were unbombarded embryos/callus. Positive controls were unbombarded embryos/callus spiked with purified enzyme. Transformed callus was then tested for ESP1 or APAO activity as previously described. Out of 67 samples tested 18 samples contained both ESP1 activity and APAO activity. Peroxisomally targeted APAO and apoplast targeted fumonisin esterase can both be successfully expressed in a plant cell.

Another preferred construct for expression of APAO in a plant is targeting the APAO to the mitochondrial membrane. A C-terminal extension is required for targeting monoamine oxidases MAO-A and MAO-B to mammalian outer mitochondrial membranes. A MAO-A, MAO-B, or functionally similar C-terminal extension can be ligated in-frame to APAO or trAPAO to facilitate localization of this enzyme to the mitochondrial membrane of maize or other transformed species.

EXAMPLE 12

Comparison of APAO Sequence With Other Sequences

The *Exophiala* cDNA APAO (SEQ ID NO: 22) contains an 1800 bp open reading frame coding for a 600 amino acid polypeptide (SEQ ID NO: 23) with divergent homology to two classes of proteins. The carboxy three-fourths of APAO (amino acids 137 to 593) is strongly homologous to flavin amine oxidases, a group of enzymes catalyzing the oxidative deamination of primary amines at carbon 1. The amine oxidase function of the carboxy terminal domain was confirmed by expression of a truncated APAO polypeptide (from 137 to 600) in both *Pichia pastoris* and *E. coli*, using AP1 as a substrate (see Example 9). The amino terminal portion of APAO, in contrast, (from approx. 5 to 134) shows significant homology to a group of small deduced open reading frames (ORFs) reported in several bacteria and blue-green algae, as well as several higher organisms. These ORFs code for small proteins of unknown function, ranging in size from 14 to 17 kDA. The juxtaposition of these divergent homologies in a single polypeptide has not been reported previously.

Flavin amine oxidases (E.C. 4.1.4.3) are a group of flavoenzymes found in both higher and lower organisms, and serve a variety of functions in catabolism. They catalyze the oxidative deamination of primary amino groups located at the C-1 position of a variety of substrates, resulting in an aldehyde product plus ammonia and hydrogen peroxide. The APAO enzymes of the present invention are the first flavin amine oxidase known to attack a primary amine not located at C-1 (i.e. C-2 of AP1) and resulting in a keto rather than aldehydic product. However, amino acid oxidases, while not closely related to flavin amine oxidases, are flavoenzymes that oxidize a C-2 amine adjacent to a C-1 carboxyl group.

The monoamine oxidases MAO A & B, (from human, bovine, and trout), are localized in the mitochondrial outer membrane of higher organisms and regulate the level of neurotransmitters. Microbial examples include a fungal amine oxidase (*Aspergillus niger* (*niger*) MAO-N) involved in amine catabolism, and a bacterial putrescine oxidase from a gram (+) bacterium (*Micrococcus rubens*.). The primary polypeptides vary in length from 478 to 527 amino acids, and share regions of high amino acid sequence conservation at the 5' end as well as at various points through the coding region. Protein alignments generated with PileUp (GCG) indicate that trAPAO contains all conserved domains found in this class of proteins including those near the 5' end.

The amine oxidase domain of trAPAO contains several key features shared by this class of enzymes, including an amino-terminal dinucleotide (ADP) binding region characterized by a beta-alpha-beta stretch containing three invariant glycines (G -X-G-X-X-G) in the beta-alpha turn. In trAPAO, this sequence is (DVVVV**G**A**G**LS**G**). This region is involved in FAD binding. Absent are several features unique to the mammalian amine oxidases, including several important cysteine residues (Wu *et al., Mol Pharm* 43:888 (1993)), one of which (Cys-406 of MAO-A) is involved in covalent binding of FAD, and a carboxy-terminal extension that has been demonstrated to be involved in transporting to and anchoring the MAO in the outer mitochondrial membrane. The *Aspergillus* enzyme MAO-N has been demonstrated to contain non-covalent FAD, and also lacks the conserved cysteine. Therefore it is possible that the APAO enzyme has a non-covalent FAD. The *Aspergillus* MAO-N has a carboxy-terminal tripeptide Ala-Arg-Leu that is involved in peroxisomal targeting and localization; this sequence is absent from *Exophiala* MAO.

The amine oxidase domain of trAPAO contains a total of seven cysteines, compared to ten for the *Aspergillus* enzyme and only two for the *Micrococcus* enzyme. The mammalian MAO enzymes contain variable numbers of cysteines (at least ten), some of which are highly conserved (including the FAD binding residue mentioned above). The trAPAO sequence also has two putative glycosylation sites (NDS, NQS) towards the amino end.

The purpose of the amino-terminal extension of APAO and the basis for its homology to a group of 14-17 kDa proteins is not clear. In *Synechocystis*, a similar polypeptide ORF is located immediately upstream of the NADP-dependent glutamine dehydrogenase (gdhA) and has been shown to be required for functional expression of gdhA (Chavez et al, 1995). However, in trAPAO the domain is clearly not necessary for enzymatic activity, as shown by the results of the expression experiments using the truncated APAO. An interesting clue comes from the frequent association of this small ORF with gene clusters involved in oxidoreductase activity in bacteria, or induced by heat stress in mice, suggesting a possible role in redox protection. A byproduct of amine oxidase activity is hydrogen peroxide. Flavoenzymes and other redox enzymes are often susceptible to inactivation by hydrogen peroxide (Schrader *et al.*, *App Microb Biotechnol* 45:458; Aguiree, *et al.*, *J Bacteriol* 171:6243 (1989)), and it is possible that this protein has a protective role against oxidants such as hydrogen peroxide. Alternatively, this domain could be involved in enzyme function, localization or association of the enzyme with other structures. No signal peptide region can be detected in this amino terminal region.

In multiple sequence alignment using GCG PileUp, trAPAO is most similar to putrescine oxidase of *Micrococcus rubens*, Swissprot accession number P40974, (30% identical amino acids, 40% similar). Homology with several mammalian monoamine oxidases A and B, Swissprot accession numbers P21397 *(Homo Sapiens* mao a), P19643 (*Rattus norvegicus* mao b), P21396 (*Rattus norvegicus* mao a), and P21398 (*Bos taurus* mao a), is somewhat less, ranging from 25 to 28% identity and 36 to 40% similarity. Homology to the only other fungal flavin amine oxidase known, MAO-N from *Aspergillus niger* (Swissprot accession number P46882), is somewhat lower (24% identical, 34% similar). The microbial enzymes are considerably divergent from each other, while the mammalian monoamine oxidases share 65 to 87% identity.

The amino terminal domain (ATD) of APAO also shows homology to a 14.5 kD protein from human and rat phagocytes that shows translational inhibition activity *in vitro* (Swissprot accession # P52758, P52759) Schmiedeknecht, *et al., Eur J Biochem* 242 (2), 339-351 (1996)), and includes a heat-responsive protein from mouse (Samuel, *et al., Hepatology* 25 (5), 1213-1222 (1997)). This suggests that this family of proteins is involved in regulating cellular metabolism. No example exists in which this domain is fused to a larger protein domain, however, making APAO unique. Without intending to be limited by theory, all of this suggests, that this domain plays a regulatory role in APAO gene expression, possibly to prevent translation of the message when it is not needed. This raises the question of how translation of the message is restored when active enzyme is required by the *Exophiala* cell. Possibly there are alternative start sites that begin downstream of the inhibitor domain; or proteolysis, complexing, degradation, or phosphorylation/ dephosphorylation of the inhibitor domain when it is not needed. The first possibility is less likely because there are no other ATG codons prior to the ATG at 122-124 that constitutes the predicted start site of APAO. The second possibility cannot be easily tested, although there is a casein kinase site in the ATD. Alternative roles for the ATD include oligomerization of the APAO protein, or anchoring the protein to some intracellular site, such as the membrane.

A parallel example of regulatory control over another flavoenzyme, human flavin monooxygenase 4 (FMO-4), by a C-terminal extention has been reported (Itagaki, *et al., J of Biol Chem* 271(33): 20102-20107 (1996)). In this case the introduction of a stop codon prior to the 81 base C-terminal extension allowed expression of active enzyme in heterologous systems. The role of the C-terminal portion was not elucidated, however. In another example, alternative splicing led to a shorter gene product that complexed with and interfered with the function of the normally spliced version (Quinet, *et al., J of Biol Chem* 268(23): 16891-16894 (1993)). In another case, an alternative splicing-generated insert in another protein led to inhibition of cell growth (Bhat, *et al., Protein Engineering* 9(8): 713-718 (1996)). In yet another variation, fas/Apo1 splicing variants prevent apoptosis, apparently through a 49 amino acid domain shared by all variants ((Papoff, *et al., J of Immunology* 156(12): 4622-4630 (1996)).

EXAMPLE 13

Making a Chimera Protein Containing Fumonisin Esterase and APAO activity in the Same Polypeptide.

The enzyme activities of fumonisin esterase and APAO can be combined in a single polypeptide by using the open reading frames together either with or without a spacer region between the two polypeptides. This creates a hybrid protein with dual enzyme activities that can be exported as a unit to the apoplast, and will allow both enzyme activities to be conveniently localized to the same area of the cell wall. The two cDNAs can be combined in either order, but the preferred method is to link them in the order NH_3-Esterase:APAO-COOH. The spacer, if present, may consist of a short stretch of amino acids such as GGGSGGGS, or a set of amino acids that comprises a protease cleavage site that can be acted on by an apoplastic protease. This would result in the production of stoichiometric amounts of both esterase and APAO enzymes in the apoplast. Alternatively, a polycystronic message could be engineered which is capable of direct translation of a downstream sequence, for example inclusion of an IRES sequence in the spacer region or a polynucleotide spacer region containing a polynucleotide cleavage site that can be recognized by RNAse or is a self-cleaving ribozyme. The length of the splice site could be of any length that ensures proper translation of the polynucleotide.

The esterase-APAO ligated protein can be made with any fumonisin esterase, including but not limited to, the fumonisin esterase from *E. spinifera* (ESP1) or fumonisin esterase from bacterium (BEST1). Since the pH range for maximum activity of BEST1 is similar to that of APAO (range 6.0 to 8.0), these may present the most effective combination in fusion form. In addition, any of the polynucleotides of the present invention, including APAO mutated to improve expression, may be used for an esterase-APAO ligation. As described in previous examples these fusion sequences can be placed in the appropriate expression vectors and used to express proteins in either bacteria or plants.

The nucleotide sequence of ESP1 contains three nucleotide differences and three corresponding amino acid differences for the ESP1 sequence disclosed in pending US application no. 08/888,950, filed July 7, 1997 and US patent no. 6,025,188, issued February 15, 2000. Both the sequences disclosed in the present application and the sequences disclosed in the pending US applications contain functional fumonisin esterase

genes. For the purposes of the present invention, either the original ESP1 sequences or the ESP1 sequences may be used in combination with the APAO sequences or in fusion sequences. The nucleotide sequence of a BAA:ESP1:trAPAO construct for plant expression can be found in SEQ ID NO: 24 and the translation in SEQ ID NO: 25. The nucleotide sequence for a BAA:BEST1:K:trAPAO construct for plant expression can be found in SEQ ID NO: 26 and the translation in SEQ ID NO: 27. The nucleotide sequence of a GST:ESP1:K:trAPAO fusion for bacterial expression in a pGEX-4T-1 or similar vector can be found in SEQ ID NO: 28 and the translation in SEQ ID NO: 29. The nucleotide sequence for a GST:BEST1:K:trAPAO fusion for bacterial expression in a pGEX-4T-1 or similar vector can be seen in SEQ ID NO: 30 and the translation in SEQ ID NO: 31.

EXAMPLE 14
APAO Substrate Studies

The following assay was used to determine the substrate specificity of the APAO enzyme. Reaction mix: 436 µl of 200 mM Na-phosphate, pH8.0; 50 µl substrate (10 mM); 2 µl Amplex Red (1 mg in 200 µl DMSO); and 2 µl of Peroxidase (5000 U/ml). The APAO enzyme was recombinant enzyme produced as GST fusion in *E. coli*, purified over a glutathione affinity column and cleaved with thrombin to remove the GST. All components were mixed at room temperature. The initial rate was determined in a spectrophotometer at 572 nm over one minute by absorbance units/second (BLANK). Ten microliters of APAO at 70 ug/ml was added and mixed. The initial rate was again determined at 572 nm over one minute in absorbance units/second (SAMPLE). The rates were converted to absorbance units/minute. The BLANK value was subtracted from the SAMPLE value. The absorbance units were converted to µM H_2O_2 wherein 1 µM H_2O_2 equals 0.138 absorbance units at pH 8.0.

SUBSTRATES FOR APAO

SUBSTRATE	RATE $\mu M\ H_2O_2/min$
1 mM Fumonisin B1	0.1429
1 mM AP1	0.8876
0.5mg/mL Fumonisin B2	0.3058
1 mM Fumonisin B3	0.1449
0.5mg/mL Fumonisin B4	0.1728
1 mM norepinephrine	0.0087
1 mM epinephrine	0.0071
1 mM dopamine	0.0040
1 mM spermine	0.0002

NOT SUBSTRATES FOR APAO (defined as compounds resulting in less than 1% conversion to hydrogen peroxide by APAO relative to AP1 under similar conditions of time, pH, temperature, and substrate concentration): 2-phenylethylamine, spermidine, EDTA-Na_2, tryptamine, putrescine, benzamidine, serotonin, cadaverine, Pefabloc SC, tyramine, 1,3-diaminopropane, leupeptin, histamine, hydroxylamine, aprotinin, deprenyl, Fumonisin C4, isoniazid, sphingosine, phenelzine, sphinganine, phytosphingosine, D-alanine, DL-alanine, L-arginine, L-asparagine, L-aspartic acid, D-aspartic acid, L-cysteine, L-glutamine, L-glutamic acid, glycine, L-histidine, L-isoleucine, L-leucine, L-lysine, DL-lysine, L-methionine, DL-methionine, L-phenylalanine, L-proline, L-threonine, L-tryptophan, L-tyrosine, L-valine.

EXAMPLE 15

Sites on APAO for Possible Mutagenesis

Some cytosolic enzymes, when engineered for secretion by fusion with a heterologous signal peptide, lack function due to glycosylation at one or more potential glycosylation sites (amino acid consensus sequence N-X-S/T) that are not normally glycosylated in the native environment (Farrell *et al.*, *Plant Mol Biol* 15(6):821-5 (1990)). Since APAO lacks a recognizable signal sequence, it may be cytoplasmically localized in *Exophiala spinifera*, although secretion by some other method not involving a signal peptide cannot be ruled out. APAO contains two potential glycosylation sites, which may be glycosylated when APAO is secreted in a plant or other eukaryotic cell. Other modifications to APAO can be made to improve its expression in a plant system, including

site-directed mutagenesis to remove selected cysteine residues, which may be detrimental to proper folding when the protein is secreted into the endomembrane system for delivery to the apoplast.

Knowledge of the 3-dimensional structure of APAO would help to evaluate the likelihood that particular amino acids could contribute to misfolding, and increase the odds of making rational changes in the APAO sequence for successful secretion. To this end a 3-dimensional model of APAO was developed based on the crystal structure of a related amine oxidase from maize, maize polyamine oxidase or MPAO (Binda *et al., Structure* 7:265-276 (1999)). The model was derived by automated modeling using the program *Modeler* (Molecular Simulations, Inc., San Diego, CA) and the resulting 3-D structure showed excellent fit based on an RMS deviation of 0.68 Å for the backbone coordinates of the two structures. The 3-D model of APAO based on MPAO is shown in Figures 1 and 2. Some of the possible mutations of APAO, which would result in removal of glycosylation sites or removal of cysteine residues can be seen below and in Figure 1.

Table of site-directed mutagenesis vectors and enzyme assay results.

Residue number	1	2	3	4	5	6	7	8	9	Glyc Site 1a,b		Glyc Site 2a,b		*E coli* expression vector, APAO or trAPAO activity		Maize expression vector, APAO or trAPAO Activity	
Residue position in APAO or trAPAO	C64	C109	C167	C292	C351	C359	C387	C461	C482	N201	S203	N204	S206				
Construct	Amino acid substitution													Plasmid	Act	Plasmid	Act
Wild type APAO-1														PHP13367	+	PHP	-
Glyc(-) 1a2a APAO										A		A		PHP16284	-	n/a	
Glyc(-) 1a2b APAO										A			A	PHP16285	-	n/a	
Glyc(-) 1b2a APAO											A	A		PHP16286	-	n/a	
Glyc(-) 1b2b APAO											A		A	PHP16287	-	n/a	
Glyc(-)2a N204A APAO												A		PHP16589	+/-	n/a	
Glyc(-)2b S206A APAO													A	PHP16590	+	PHP16711	-
Cys(-)#8 trAPAO								S						PHP16737	+	n/a	
Cys(-)#6,8 trAPAO						S		S						PHP16738	+		
Cys(-)#3,6,8 trAPAO			S			S		S						PHP17089	+[1]		
Cys(-)#1,2,7 APAO	A	A					A										

A= alanine
S= serine
1 - activity against FB1 equals wild type; activity against AP1 was reduced.

APAO and trAPAO polypeptide sequence, annotated. (SEQ ID NO: 47)
The amino terminal domain is italicized. Cysteines and residues involved in putative glycosylation sites are underlined. Boxed residues represent amino acids that were successfully altered without complete loss of activity as E coli-expressed protein.

MALAPSYINPPNVASPAGYSHVGVGPDGGRYVTIAGQIGQDASGVTDPAYEKQVAQAFA
NLRACLAAVGATSNDVTKLNYYIVDYAPSKLTAIGDGLKATFALDRLPPCTLVPVSALSSP

*EYLFEVDATALVPGHTTP*DNVADVVVVGAGLSGLETARKVQAAGLS$\boxed{C}$LVLEAMD
RVGGKTLSVQSGPGRTTINDLGAAWINDS$\boxed{N}$Q$\boxed{S}$EVSRLFERFHLEGELQRTTGNSIH
QAQDGTTTTAPYGDSLLSEEVASALAELLPVWSQLIEEHSLQDLKASPQAKRLDSV
SFAHYCEKELNLPAVLGVANQITRALLGVEAHEISMLFLTDYIKSATGLSNIFSDKK
DGGQYMRCKTGMQSI$\boxed{C}$HAMSKELVPGSVHLNTPVAEIEQSASGCTVRSASGAVF
RSKKVVVSLPTTLYPTLTFSPPLPAEKQALAENSILGYYSKIVFVWDKPWWREQGF
SGVLQSS$\boxed{C}$DPISFARDTSIDVDRQWSITCFMVGDPGRKWSQQSKQVRQKSVWDQL
RAAYENAGAQVPEPANVLEIEWSKQQYFQGAPSAVYGLNDLITLGSALRTPFKSV
HFVGTETSLVWKGYMEGAIRSGQRGAAEVVASLVPAA

APAO enzyme activity is maintained when a serine residue at position 206 is mutated to alanine, eliminating a potential glycosylation site (N204 – S206) close to the putative substrate binding site. Please see the tables entitled "Table of site-directed mutagenesis vectors and enzyme assay results" and "Glyc(-) APAO lysates from *E. coli*." The polynucleotide sequence of APAO mutated to alter the serine at position 206 to an alanine (S206A) can be seen in SEQ ID NO: 32. The resulting polypeptide is shown in SEQ ID NO: 33.

Glyc(-) APAO lysates from *E coli*

Sample (lysate)	Substrate	M H_2O_2/min[1]	Conclusion
WT APAO	AP1 FB1	1.92 0.12	Active (wild type) Slightly active (wt)
N204A	AP1 FB1	0.09 0.04	Slightly active Slightly active
S206A	AP1 FB1	0.85 0.07	Partially Active Slightly active

However, in transient expression assays in maize, expression of S206A resulted in no detectable enzyme activity. Please see the table above entitled "Table of site-directed mutagenesis vectors and enzyme assay results." Thus, elimination of this glycosylation site is not in itself sufficient to have an active protein upon secretion. This could be due to glycosylation occurring at a second adjacent site (N201 - S203). However, no active APAO was recovered when either N201 or S203 is mutated along with S206. Please see the table entitled "Table of site-directed mutagenesis vectors and enzyme assay results."

While not to be limited by theory, the molecule may be inactive because both N201 and S203 are buried within the tertiary structure of APAO, and any modification of side chains disrupts proper folding or conformation, or FAD binding. This is backed up by predicted solvent accessibility numbers for these residues in the 3-D model based on the

maize amine oxidase. Please see the table below entitled "Solvent accessibility for cysteine residues of truncated APAO." The elimination of APAO glycosylation site at amino acids 204 to 206 is not sufficient to allow APAO to be secreted from the cell and retain full enzyme activity, but elimination of this site may improve chances for obtaining a fully active enzyme once the other roadblock(s) to secretability have been resolved. In other words, elimination of this site may be necessary but not sufficient to produce active secretable APAO.

APAO also contains nine cysteine residues, which are likely to be unpaired in the reducing environment of the cytosol but which may crosslink unfavorably upon secretion. Cysteines are present at residues 64, 109, 167, 292, 351, 359, 387, 461, and 482. The 3-D model helps predict the relative location of each amino acid in the structure, and whether it is solvent accessible or buried. Buried residues are more difficult to mutate without destroying structural integrity.

Solvent accessibility for cysteine residues of truncated APAO

APAO Position[1]		Position[2]	Cys#[3]	aa MPAO		-1	0	1	average	Conclusion
Cys	26	167	3	Leu	32	0.675	0.253	0.24	**0.389333**	maybe partially exposed
Cys	151	292	4	Asn	147	0.069	0.122	0.147	**0.112667**	buried
Cys	210	351	5	Tyr	211	0.184	0.244	0.03	**0.152667**	buried
Cys	218	359	6	Thr	219	0.633	0.319	0.447	**0.466333**	maybe partially exposed
Cys	246	387	7	Val	247	0.145	0.046	0.366	**0.185667**	buried
Cys	320	461	8	Ser	324	0.199	0.789	0.643	**0.543667**	exposed
Cys	341	482	9	Leu	346	0.152	0.071	0.052	**0.091667**	buried

1. Relative to amino acid 1 of truncated APAO
2. Relative to amino acid 1 of full length APAO
3. Cysteine number relative to full length APAO

Proteins that are secreted to the apoplast are folded to their mature form in the highly oxidizing environment of the ER/Golgi. Among other things this promotes crosslinking of cysteine residues often found in secreted proteins. Unpaired cysteines that are solvent-accessible are rare in secreted proteins, since they would rapidly be oxidized by other cysteine residues of the same protein or another protein. Although not to be limited by theory, it is possible that APAO is normally a cytosolic protein, and thus the presence of nine cysteine residues would not be unusual even though they may not be crosslinked in

the mature protein. In fact, the 3-D model predicts that they would not be crosslinked because the intermolecular distances predicted would be too great. Therefore it is possible that secretion of APAO to the apoplast results in an improper folding and crosslinking of cysteines in the Golgi, and results in inactive enzyme. Using the solvent accessibility tables from APAO modeled against MPAO, the three most solvent-exposed cysteines were identified and then eliminated by site-directed mutagenesis of the APAO cDNA. The sequence of APAO mutated at cysteine 461 and used for expression in bacteria can be seen in SEQ ID NO: 48. The resulting protein is shown in SEQ ID NO: 49. The polynucleotide and resulting polypeptide sequence of APAO mutated at both cysteines 359 and 461 and used for in the bacterial expression system can be seen in SEQ ID NOS: 50 and 51. The polynucleotide and resulting polypeptide sequence of APAO mutated at cysteines 169, 359, and 461 can be seen in SEQ ID NOS: 52 and 53.

The APAO molecules mutated at specific cysteines were tested in a bacterial expression system using the previously described Amplex Red assay. The results can be seen in the table below entitled "Cys(-) APAO lysates from *E. coli*." The mutated APAO molecules can then be tested in maize, linked to a signal peptide, as previously described. Either one of the cysteines or two or three together could be mutated to serines without any measured loss in APAO enzyme activity of the *E coli*-expressed enzyme. In fact, one of the *E coli*-expressed clones (C359S + C461S; PHI16738) had more APAO activity in crude lysates than wild type enzyme and may represent a catalytic improvement. A triply Cys-mutated version of APAO does not show catalytic improvement but retains full activity of the wild type enzyme against FB1, although AP1 activity was somewhat reduced. The mutated versions of APAO operably linked to a signal sequence, which retain function when expressed as recombinant fusion proteins in *E. coli*, may also provide additional stability or foldability when expressed in plants or other secretion expression systems.

Cys(-) APAO lysates from *E coli*

Sample (lysate)	Substrate	M H_2O_2/min^1	Conclusion
WT APAO	AP1 FB1	2.14 0.11	Active (wild type) Slightly active (wt)
C461S	AP1 FB1	2.25 0.14	Fully Active Slightly active
C359S, C461S	AP1 FB1	3.90 0.16	Fully/More Active Slightly active
C167S, C359S, C461S	AP1 FB1	0.27 0.25	Slightly active Slightly active

Triple Cys(-) APAO lysates from *E coli*

Sample (lysate)	Substrate	M H_2O_2/min^1	Conclusion
WT APAO	AP1 FB1	1.16 0.27	Active (wild type) Slightly active (wt)
C167S, C359S, C461S	AP1 FB1	0.27 0.26	Slightly Active Slightly active

It is expected that the S206A mutations will contribute to the functionality of secreted APAO by reducing the degree of glycosylation and the C167S, C359S, and C461S mutations (or combinations thereof) will improve the functionality of secreted APAO by reducing chances for spurious disulfide formation on folding.

To determine expression of a mutated APAO in maize, three APAO constructs were introduced into maize embryos by Agrobacterium-mediated transformation (Zhao et al, 1999, US Patent 5,981,840). The three constructs were PHP17105 (Ubi:BAA:Cys(-)K-trAPAO (C359S, C461S):PinII), PHP17108 (Ubi:Cys(-)K-trAPAO (C359S, C461S):PinII), and PHP17110 (Ubi:APAO:PinII). In addition, PHP16543 (NOS:CRC:PinII-Ubi:MO-PAT:T35) was introduced as a negative control and PHP15258 (Ubi:APAO:PinII-Ubi:BAA:ESP1:PinII-P35S:PAT:T35S) was introduced as a non-targeted positive control. One experiment with two replications was performed. Samples were assayed for both APAO activity by TLC as described previously and by Enzyme Linked ImmunoSorbent Assay (ELISA). For a discussion of ELISA methods, please see, for example, *Current Protocols in Molecular Biology*, 2:11.1.1-11.3.4, John Wiley & Sons, Inc. (Ausubel, *et al.*, eds. 1994). The APAO ELISA is a capture format assay for the quantitative determination of APAO protein in the presence of extracted maize tissue protein. It was performed by co-incubation of biotinylated antibody with an extract prepared from leaf, seed, or callus in phosphate buffered saline with 0.5% Tween-20®. The detection of the antibody complex was accomplished through the added incubation of

streptavidin-alkaline phosphatase (Bio-Rad Life Sciences Products #19542-018), followed by the addition of substrate (pNPP tablets, Sigma #104-105). The resultant color intensity was quantified by determining optical density and was directly proportional to the amount of APAO protein present in the sample extracts. The assay has no matrix effects at 1μg/well or below for maize leaf, seed, or callus protein. The standard curve was spiked with wild type extract at levels above 1.0 μg/well. The transient testing results are summarized in the table below.

Transient Testing of APAO Constructs (6-8-2000)

Experiment	Rep	Construct	APAO-TLC	APAO-ELISA (ppm)
negative control	none	none	0	-2
4350.08.01	1	php16543, as a (-) control	0	-4
4350.08.02		php15258, non-targeted APAO as a (+) control	3	out high
4350.08.03		php17105, UBI-BAA::CYS(-)K-TR-APAO (C359S, C461S)	1	107
4350.08.04		php17108, UBI-CYS(-)K-TR-APAO (C359S, C461S)	3	270
4350.08.05		php17110, UBI-APAO	3	out high
4350.08.06	2	php16543	0	-5
4350.08.07		php15258	3	313
4350.08.08		php17105	0	52
4350.08.09		php17108	2	143
4350.08.10		php17110	2	123
3477.27.01	transformed callus lines	php15258 as postive controls	1	118
3477.27.02			2	141
3477.27.03			2	187
3477.27.04			2	184

As can be seen in the Table above, the BAA-targeted APAO (PHP17105) did not accumulate as much APAO as the non-BAA targeted counterpart (PHP17108). Although not to be limited by theory, the lack of APAO protein accumulation rather than APAO function may play a role in the lack of detectable APAO activity with the BAA-targeted APAO construct. It appears that only when the APAO concentration exceeds 100 ppm can APAO activity be seen by TLC. Nevertheless, the double Cys(-) mutant is active in maize when expressed either cytosolically or extracellularly.

EXAMPLE 16

Other APAO Polynucleotides From *Exophiala spinifera* and *Rhinocladiella atrovirens*

Using primers designed from the APAO isolated from *Exophiala spinifera*, ATCC 74269(Table 15), three new APAO polynucleotides were isolated from *Exophiala spinifera* (isolates ESP002 and ESP003), designated ESP002_C2, ESP002_C3 and ESP003_C12

and three new APAO polynucleotides from *Rhinocladiella atrovirens* (isolate RAT011) designated RAT011_C1, RAT011_C2, RAT011_C4. The strains used to isolate the polynucleotides are described below.

Isolate	Genus species	Source	FB1 degrader	APAO homologs isolated
ESP002	*Exophiala spinifera*	Palm, ATCC 26089	Yes	ESP002_c2 in pGEX4T1 ESP002_c3 in pGEX4T1
ESP003	*Exophiala spinifera*	Maize seed	Yes	ESP003_c12 in pGEX4T1
RAT011	*Rhinocladiella atrovirens*	Maize seed	Yes	RAT011_c1 in pGEM11Zf+ RAT011_c2 in pGEX4T1 RAT011_c4 in pGEM11Zf+

Growth conditions and production of culture material

1. Streak 150 x 15 mm YPD plates with a glycerol aliquot of the above fungal isolates.
2. Grow at 28° C in the dark until there is sufficient growth for inoculating liquid medium usually at least two weeks.
3. Mycelia and spores were scraped from the plates or agar cubes used to inoculate 50 mls YPD broth in 250 ml baffled flasks.
4. Flasks of culture material were grown at 28° C in the dark at ~125 rpm.
5. After sufficient growth was obtained the cultures were harvested by pelleting the culture in 50 ml centrifuge tubes at 3400 rpm for 15 min.
6. The supernatant was discarded and the pellets were frozen at -20° C.

YPD broth and agar medium

Amount per liter:	Yeast Extract	10 g	
	Bactopeptone	20 g	
	Dextrose	0.5 g	
	Bactoagar	15 g	(for agar media only)

DNA Isolation,

The DNA was isolated according to a modified version of a plant CTAB DNA extraction protocol (Saghai-Maroof MA, *et al., Proc Natl Acad Sci, USA*, 81:8014-8018 (1984)) as follows.

1. Place 0.2-0.5 g (dry weight) lyophilized fungal mycelium in a 50 ml disposable centrifuge tube, break up mat with a spatula or glass rod. Shake briefly.
2. Add 10 ml (per 0.5 g mat) of CTAB extraction buffer. Gently mix to wet all the powdered mat.

3. Place in 65° C water bath for 30 minutes.
4. Cool. Add an equal volume of phenol:chloroform. Shake briefly to mix.
5. Centrifuge 20 minutes at 3400 rpm.
6. To the aqueous phase add an equal volume of chloroform:isoamyl alcohol (24:1). Shake briefly to mix.
7. Centrifuge 15 minutes at 3400 rpm.
8. To aqueous phase add an equal volume of isopropanol.
9. Centrifuge for 30 minutes at 3400 rpm to pellet precipitated DNA.
10. Rinse DNA pellet with 70% ethanol.
11. Air dry pellet.
12. Resuspend pellet in 1-5 ml TE containing 20 ug/ml RNase A.

CTAB Extraction Buffer

0.1 M Tris, pH 7.5
1% CTAB (mixed hexadecyl trimethyl ammonium bromide)
0.7 M NaCl
10 mM EDTA
1% 2-mercaptoethanol
Add proteinase K to a final concentration of 0.3 mg/ml prior to use.

Primer Design

Primers used were gene specific primers based on APAO polynucleotide sequence (SEQ ID NO: 22) with restriction enzymes sites for cloning. The 5'-primer, 26194, contained the restriction enzyme recognition site, EcoRI. The complementary 3'-primer, 23259, contained the restriction enzyme recognition site, NotI.

26194
5' ggggaattcATGGCACTTGCACCGAGCTACATCAATC 3' , 37-mer (SEQ ID NO: 34)

23259
5' gggGCGGCCGCCTATGCTGCTGGCACCAGGCTAG 3' , 34-mer (SEQ ID NO: 13)

PCR conditions

1. The PCR cocktail: per 50 ul reaction per 0.2 ml tube

10 mM dNTPs	1 ul
10X Advantage polymerase buffer	5 ul
HPLC water	38 ul
10 uM primer 26194	2 ul
10 uM primer 23259	2 ul
50 X Advantage polymerase mix (Clontech)	1 ul
Template, genomic DNA, 50 ng/ul	1 ul

2. Thermocycling conditions:
MJ PTC-100 AgV Thermocycler:

Step		
1	95°	2 minutes
2	95°	30 seconds
3	60°	1 minute
4	72°	1 minute 30 seconds
5	Go to step 2, 34X more	
6	72°	5 minutes
7	4°	Hold
8	End	

3. PCR products were analyzed on a 1% LE-agarose, TAE plus ethidium bromide gel.
Bands of about 1900 bp were seen on the gel. The band was not present in the no DNA control reaction.

Cloning Protocols

1. DNA was extracted from excised gel fragments using a QIAGEN Gel Extraction Kit (Catalog number 28704, QIAGEN, Santa Clara, CA).
2. PCR fragments were digested with EcoRI and Not I to free up the sites for cloning into EcoRI and Not I digested vector, either pGEX4T1 (Phamacia) or pGEM11Zf+ (Promega).
3. Digests were cleaned up and desalted used a QIAquick PCR Purification Kit (Catalog number 28104).
4. Isolated fragment was quantified and checked for purity on a 1% LE-agarose, TAE + ethidium bromide gel.
5. Fragments were ligated into compatible sites in either pGEX4T1 (Phamacia) or pGEM11Zf+ (Promega).
6. After heat inactivation Library efficiency DH5 competent *E. coli* were transformed with a small amount of the ligation reaction.
7. LB + carbenicillin, 50 ug/ml, plates were spread with an aliquot of the transformation mix, grown overnight at 37° C.
8. Colonies were screened for full-length insert using a PCR miniprep method utilizing vector primers flanking the multiple cloning region.

9. Positive clones were identified and overnight cultures grown for plasmid isolation and verification by sequencing.

10. Positive clones are identified as follows:

DH5 :pGEX4T1:ESP002FL_c2	(from palm tree isolate)
DH5 :pGEX4T1:ESP002FL_c3	(from palm tree isolate)
DH5 :pGEX4T1:ESP003FL_c12	(from maize isolate)
DH5 :pGEM11Zf+:RAT011FL_c1	(from maize isolate)
DH5 :pGEM11Zf+:RAT011FL_c4	(from maize isolate)
DH5 :pGEX4T1:RAT011FL_c2	(from maize isolate)

**Important note: These are genomic clones containing two introns

Sequence Results

Three APAO polynucleotides and related polypeptides were isolated from *Exophiala spinifera* (isolates ESP002 and ESP003), designated ESP002_C2, (SEQ ID NOS: 35 and 36) ESP002_C3 (SEQ ID NOS: 37 and 38) and ESP003_C12 (SEQ ID NOS: 39 and 40). Three APAO polynucleotides were isolated from *Rhinocladiella atrovirens* (isolate RAT011) designated RAT011_C1 (SEQ ID NOS: 41 and 42), RAT011_C2 (SEQ ID NOS: 43 and 44), and RAT011_C4 (SEQ ID NOS: 45 and 46). Introns were detected by comparison of the genomic sequence with the cDNA sequence of APAO from *E. spinifera* 2141.10 (SEQ ID NO: 22), and by identifying putative intron splice junctions in the gap domains (Shah, *et al., Journal of Molecular and Applied Genetics* 2:111-126 (1983)).

Plasmids containing the polynucleotide sequences of the invention were deposited with American Type Culture Collection (ATCC), Manassas, Virginia, and assigned Accession No. 98812, 98813, 98814, 98815, 98816, (all deposited on July 15, 1998) and PTA-32 (deposited on May 7, 1999). The deposits will be maintained under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure. The deposits were made merely as a convenience for those of skill in the art and are not an admission that a deposit is required under 35 U.S.C. § 112.

Preliminary sequence results were entered into GCG, and nucleotide and protein alignments were done in a pileup using a software program called Genedoc for shading and homology comparisons (Nicholas, *et al., EMBNEW.NEWS* 4:14 (1997; or at the Internet site http://www.cris.com/~Ketchup/genedoc.shtml). The first APAO (SEQ ID NO: 22) sequence was included for comparison. Comparing the reference sequence SEQ ID NO:

22 to the other homologs sequence identities range from 96 to 99% (identities are lower since APAO introns were not included). Homologies are slightly higher comparing *Exophiala* genes sequences. At the amino acid sequence level the comparison of the reference sequence (SEQ ID NO: 23) to the other homologs yielded sequence identities of approximately 97%.

All publications and patent applications in this specification are indicative of the level of ordinary skill in the art to which this invention pertains. All publications and patent applications are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated by reference.

The invention has been described with reference to various specific and preferred embodiments and techniques. However, it should be understood that many variations and modifications may be made while remaining within the spirit and scope of the invention.

WHAT IS CLAIMED IS:

1. An isolated polynucleotide comprising an APAO encoding polynucleotide linked to a fumonisin esterase encoding polynucleotide, wherein the APAO encoding polynucleotide comprises a member selected from:

a) a polynucleotide encoding a polypeptide selected from SEQ ID NOS: 6, 11, 23, 33, 36, 38, 40, 42, 44, 46, 49, 51 and 53;

b) a polynucleotide having at least 70% sequence identity to a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 33, 35, 37, 39, 41, 43, 45, 48, 50 and 52; and

c) a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 32, 35, 37, 39, 41, 43, 45, 48, 50 and 52.

2. A recombinant expression cassette comprising a polynucleotide of claim 1 operably linked to a promoter.

3. The recombinant expression cassette of claim 2 wherein the polynucleotide is operably linked to a plant signal sequence.

4. A vector comprising the recombinant expression cassette of claim 2.

5. A host cell comprising the recombinant expression cassette of claim 2.

6. The host cell of claim 5 wherein the cell is a plant cell.

7. The host cell of claim 6 wherein the plant cell is selected from the group consisting of maize, sorghum, wheat, tomato, soybean, alfalfa, sunflower, canola, cotton, barley, millet, and rice.

8. A plant comprising a polynucleotide of claim 1.

9. A seed from a plant of claim 7.

10. An isolated polypeptide comprising a member selected from:

a) a polypeptide comprising at least 70% sequence identity to a polypeptide selected from SEQ ID NOS: 6, 11, 23, 33, 36, 38, 40, 42, 44, 46, 49, 51 and 53;

b) a polypeptide encoded by a polynucleotide having at least 70% sequence identity to a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 32, 35, 37, 39, 41, 43, 45, 48, 50 and 52; and

c) a polypeptide selected from SEQ ID NOS: 6, 11, 23, 33, 36, 38, 40, 42, 44, 46, 49, 51 and 53.

11. The polynucleotide of claim 1 wherein the fumonisin esterase encoding polynucleotide is ESP1.

12. The polynucleotide of claim 11 wherein the polynucleotide is set forth in SEQ ID NO: 24.

13. The polynucleotide of claim 1 wherein the fumonisin esterase encoding polynucleotide is BEST1.

14. The polynucleotide of claim 13 wherein the polynucleotide is set forth in SEQ ID NO: 26.

15. A method of degrading fumonisin, a structurally related mycotoxin, a fumonisin breakdown product, or a breakdown product of a structurally related mycotoxin comprising the steps of:

a) applying an APAO enzyme as a spray or wash; and

b) under degradation conditions allowing sufficient time for the polypeptide to degrade the fumonisin, the structurally related mycotoxin, the fumonisin breakdown product, or the breakdown product of a structurally related mycotoxin.

16. The method of claim 15 wherein the fumonisin or structurally related mycotoxin is present in harvested grain.

17. The method of claim 15 wherein degradation occurs during processing of the harvested grain.

18. The method of claim 17 wherein the harvested grain is to be used as animal feed.

19. The method of claim 15 wherein degradation occurs in silage.

20. The method of claim 15 wherein fumonisin esterase is also added at or before step (a).

21. The method of claim 15 wherein the APAO enzyme is selected from:

a) a polypeptide comprising at least 70% sequence identity to a polypeptide selected from SEQ ID NOS: 6, 11, 23, 33, 36, 38, 40, 42, 44, 46, 49, 51 and 53;

b) a polypeptide encoded by a polynucleotide having at least 70% sequence identity to a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 32, 35, 37, 39, 41, 43, 45, 48, 50 and 52; and

c) a polypeptide selected from SEQ ID NOS: 6, 11, 23, 36, 38, 40, 42, 44, 46, 49, 51 and 53.

22. A method of identifying transformed plant cells comprising the steps of:

a) introducing into a plant cell at least one copy of an expression cassette comprising an APAO encoding polynucleotide;

b) placing the plant cell on culture media containing an AP1 or a phytotoxic analog; and

c) identifying transformed cells as the surviving cells in the culture.

23. The method of claim 22 wherein the APAO encoding polynucleotide comprises a polynucleotide having at least 70% sequence identity to a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 32, 35, 37, 39, 41, 43, 45, 48, and 50.

24. The method of claim 22 wherein a fumonisin esterase encoding polynucleotide is also introduced into the plant cell.

25. A method of detecting fumonisins or structurally related toxins, the method comprising:

a) adding APAO enzymes to a sample containing fumonisin or a structurally related toxin;

b) reacting the sample under conditions of time and temperature sufficient to convert the toxin to the corresponding oxidized or deaminated toxin; and

c) detecting the hydrogen peroxide or ammonia produced.

26. The method of claim 25 wherein the APAO enzyme is encoded by a polynucleotide having at least 70% sequence identity to a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 32, 35, 37, 39, 41, 43, 45, 48, and 50.

27. The method of claim 25 wherein fumonisin esterase is added at or before step (a).

28. A method of producing a plant capable of degrading fumonisin, a structurally related mycotoxin, a fumonisin breakdown product, or a breakdown product of a structurally related mycotoxin comprising the steps of:

a) introducing into a plant cell at least one copy of an expression cassette comprising a polynucleotide encoding an APAO enzyme operably linked to a promoter; and

b) under degradation conditions expressing the APAO enzyme for a time sufficient to degrade the fumonisin, the fumonisin breakdown product, the structurally related mycotoxin, AP1, or a breakdown product of a structurally related mycotoxin.

29. The method of claim 28 wherein a polynucleotide encoding a fumonisin esterase is also introduced.

30. The method of claim 28 wherein the APAO enzyme is encoded by a polynucleotide having at least 70% sequence identity to a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 32, 35, 37, 39, 41, 43, 45, 48, 50 and 52.

31. The method of claim 28 wherein the plant cell is regenerated into a plant.

32. The method of claim 28 wherein a fumonisin esterase encoding polynucleotide is also introduced.

33. A host cell comprising an APAO encoding polynucleotide and a fumonisin esterase encoding polynucleotide.

34. The host cell of claim 33 wherein the APAO encoding polynucleotide comprises a polynucleotide having at least 70% identity to a polynucleotide selected from SEQ ID NOS: 5, 10, 22, 32, 35, 37, 39, 41, 43, 45, 48, and 50.

35. The host cell of claim 33 wherein the fumonisin esterase encoding polynucleotide is selected from ESP1 and BEST1.

36. The host cell of claim 33 wherein the cell is a plant cell.

37. The host cell of claim 36 wherein the cell is selected from maize, sorghum, wheat, tomato, soybean, alfalfa, sunflower, canola, cotton, and rice.

38. The host cell of claim 37 wherein the plant cell is regenerated into a plant.

39. A method of predicting possible mutagenesis sites on APAO comprising the steps of:

a) developing a 3-dimensional model of APAO; and
b) identifying sites on APAO to mutate by evaluating the likelihood that particular amino acids could contribute to misfolding.

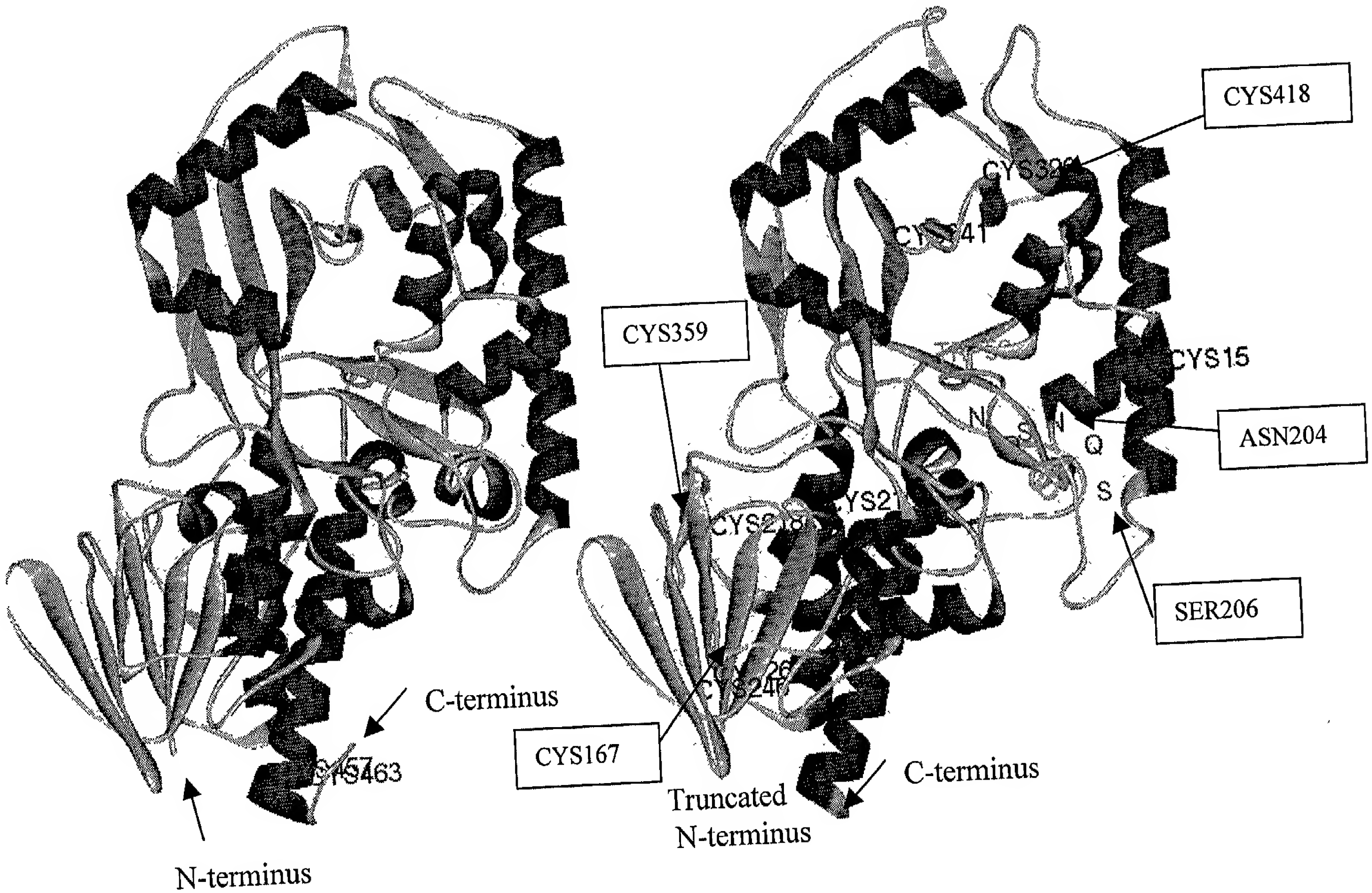
40. A 3-dimensional model of APAO generated by an automated modeling program.

41. The model of claim 40 wherein the automated modeling program is *Modeler*.

AMINO POLYOL AMINE OXIDASE POLYNUCLEOTIDES AND RELATED POLYPEPTIDES AND METHODS OF USE

Abstract

The present invention provides polynucleotides and related polypeptides of the enzyme APAO isolated from *Exophiala spinifera* and *Rhinocladiella atrovirens*. The polynucleotides may be mutated to remove glycosylation sites and cysteine residues. Additionally, the present invention provides recombinant expression cassettes, host cells, transgenic plants, and transgenic seed. The present invention also provides for polynucleotides containing both APAO and a fumonisin esterase. In addition, the present invention provides methods for producing the APAO enzyme in both prokaryotic and eukaryotic systems, methods for detecting fumonisins, and methods for identifying transformed plant cells. Methods for degrading fungal toxins in plants, grain, grain processing, silage, food crops and in animal feed are also disclosed.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N-terminus
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Truncated
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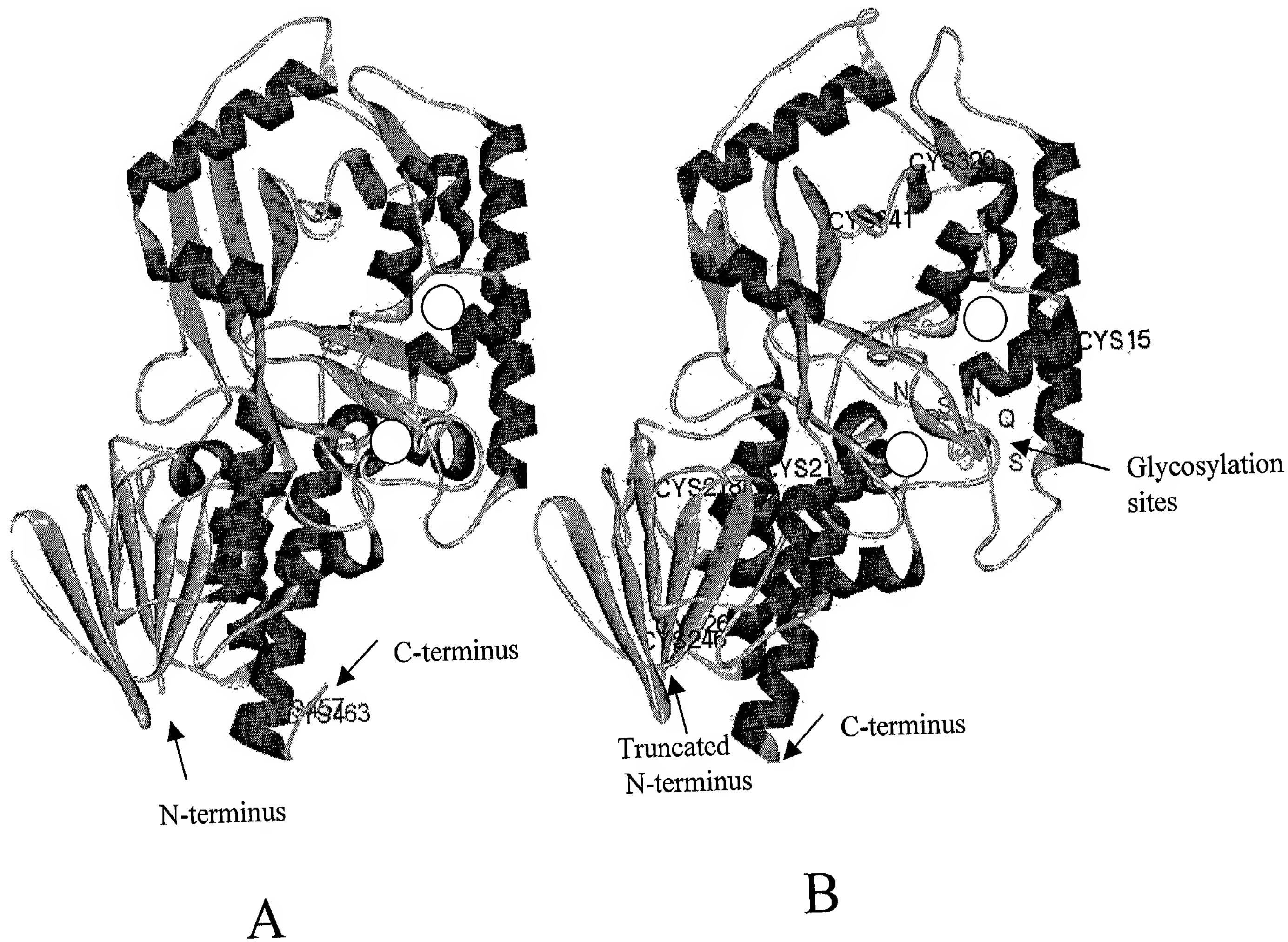
C-terminus

A

B

Figure 1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C-terminus
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B

Figure 2

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Maddox, Joyce R.
Rao,Aragula Gururaj
Crasta, Oswald R.
Folkerts, Otto

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Polynucleotides and Related Polypeptides and Methods of Use

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Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn Leu
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            180   185   190

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Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly
165 170 175
Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys
180 185 190
Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly
195 200 205
Gln Tyr Val Arg Cys Lys Thr Gly Ala Cys Gly Val Val Ser Gly Gly
210 215 220
Gly Leu Val Ser Gln Trp Ser Phe Gln Val Cys Ser Arg Phe Ala Met
225 230 235 240
Pro Cys Gln Arg Asn Leu Phe Gln Ala Gln Cys Thr Ser Thr Pro Pro
245 250 255
Ser Leu Lys Leu Ser Ser Arg His Pro Ala Val Gln Tyr Asp Arg Pro
260 265 270
Arg Ala Pro Cys Ser Glu Ala Lys Arg Trp Trp Phe Arg Tyr Arg Gln
275 280 285
Pro Cys Ile Pro Pro His Phe His His Leu Phe Pro Pro Arg Ser Lys
290 295 300
His Trp Arg Lys Ile Leu Ser Trp Ala Thr Ile Ala Arg Ser Ser Tyr
305 310 315 320
Gly Thr Ser Arg Gly Gly Ala Asn Lys Ala Ser Arg Ala Ser Ser Asn
325 330 335
Arg Ala Val Thr Pro Ser His Leu Pro Glu Ile Pro Ala Ser Thr Ser
340 345 350
Ile Asp Asn Gly Pro Leu Pro Val Ser Trp Ser Glu Thr Arg Asp Gly
355 360 365
Ser Gly Pro Asn Ser Pro Ser Arg Tyr Asp Lys Ser Leu Ser Gly Thr
370 375 380
Asn Ser Ala Gln Pro Thr Arg Thr Pro Gly Pro Lys Ser Gln Ser Arg
385 390 395 400
Pro Thr Cys Ser Lys Ser Ser Gly Arg Ser Ser Ser Ile Ser Lys Glu
405 410 415
Leu Arg Ala Pro Ser Met Gly Thr Ile Ser Ser His Trp Val Arg Arg
420 425 430
Ser Glu Arg Arg Ser Arg Val Phe Ile Ser Leu Glu Arg Arg Arg Leu
435 440 445
Phe Gly Lys Gly Ile Trp Lys Gly Pro Tyr
450 455

<210> 10
<211> 1392
<212> DNA
<213> Exophiala spinifera

<220>
<221> CDS
<222> (1)...(1389)

<221> misc_feature
<222> (1)...(3)
<223> Extra lysine in K:trAPAO

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<400> 10
aaa gac aac gtt gcg gac gtg gta gtg gtg ggc gct ggc ttg agc ggt       48
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1               5                   10                  15

ttg gag acg gca cgc aaa gtc cag gcc gcc ggt ctg tcc tgc ctc gtt       96
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val
            20                  25                  30

ctt gag gcg atg gat cgt gta ggg gga aag act ctg agc gta caa tcg      144
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
        35                  40                  45

ggt ccc ggc agg acg act atc aac gac ctc ggc gct gcg tgg atc aat      192
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
    50                  55                  60

gac agc aac caa agc gaa gta tcc aga ttg ttt gaa aga ttt cat ttg      240
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65                  70                  75                  80

gag ggc gag ctc cag agg acg act gga aat tca atc cat caa gca caa      288
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
                85                  90                  95

gac ggt aca acc act aca gct cct tat ggt gac tcc ttg ctg agc gag      336
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
            100                 105                 110

gag gtt gca agt gca ctt gcg gaa ctc ctc ccc gta tgg tct cag ctg      384
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
        115                 120                 125

atc gaa gag cat agc ctt caa gac ctc aag gcg agc cct cag gcg aag      432
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
    130                 135                 140

cgg ctc gac agt gtg agc ttc gcg cac tac tgt gag aag gaa cta aac      480
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145                 150                 155                 160

ttg cct gct gtt ctc ggc gta gca aac cag atc aca cgc gct ctg ctc      528
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
                165                 170                 175

ggt gtg gaa gcc cac gag atc agc atg ctt ttt ctc acc gac tac atc      576
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
            180                 185                 190

aag agt gcc acc ggt ctc agt aat att ttc tcg gac aag aaa gac ggc      624
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
        195                 200                 205

ggg cag tat atg cga tgc aaa aca ggt atg cag tcg att tgc cat gcc      672
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala
    210                 215                 220

atg tca aag gaa ctt gtt cca ggc tca gtg cac ctc aac acc ccc gtc      720
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225                 230                 235                 240
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gct gaa att gag cag tcg gca tcc ggc tgt aca gta cga tcg gcc tcg 768
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
245 250 255

ggc gcc gtg ttc cga agc aaa aag gtg gtg gtt tcg tta ccg aca acc 816
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
260 265 270

ttg tat ccc acc ttg aca ttt tca cca cct ctt ccc gcc gag aag caa 864
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
275 280 285

gca ttg gcg gaa aat tct atc ctg ggc tac tat agc aag ata gtc ttc 912
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
290 295 300

gta tgg gac aag ccg tgg tgg cgc gaa caa ggc ttc tcg ggc gtc ctc 960
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305 310 315 320

caa tcg agc tgt gac ccc atc tca ttt gcc aga gat acc agc atc gac 1008
Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
325 330 335

gtc gat cga caa tgg tcc att acc tgt ttc atg gtc gga gac ccg gga 1056
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
340 345 350

cgg aag tgg tcc caa cag tcc aag cag gta cga caa aag tct gtc tgg 1104
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
355 360 365

gac caa ctc cgc gca gcc tac gag aac gcc ggg gcc caa gtc cca gag 1152
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
370 375 380

ccg gcc aac gtg ctc gaa atc gag tgg tcg aag cag cag tat ttc caa 1200
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
385 390 395 400

gga gct ccg agc gcc gtc tat ggg ctg aac gat ctc atc aca ctg ggt 1248
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
405 410 415

tcg gcg ctc aga acg ccg ttc aag agt gtt cat ttc gtt gga acg gag 1296
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
420 425 430

acg tct tta gtt tgg aaa ggg tat atg gaa ggg gcc ata cga tcg ggt 1344
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
435 440 445

caa cga ggt gct gca gaa gtt gtg gct agc ctg gtg cca gca gca 1389
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala
450 455 460

tag 1392

<210> 11
<211> 463
<212> PRT
<213> Exophiala spinifera

<400> 11

Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1 5 10 15
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val
20 25 30
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
35 40 45
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
50 55 60
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65 70 75 80
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
85 90 95
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
100 105 110
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
115 120 125
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
130 135 140
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145 150 155 160
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
165 170 175
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
180 185 190
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
195 200 205
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala
210 215 220
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225 230 235 240
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
245 250 255
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
260 265 270
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
275 280 285
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
290 295 300
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305 310 315 320
Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
325 330 335
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
340 345 350
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
355 360 365
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
370 375 380
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
385 390 395 400
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
405 410 415
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
420 425 430
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
435 440 445
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala
450 455 460

<210> 12

<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer sequence designed for cloning DNA into
expression vectors, N23256

<400> 12
ggggaattca aagacaacgt tgcggacgtg gtag 34

<210> 13
<211> 34
<212> DNA
<213> Artificial Sequence

<220>
<223> Primer sequence designed for cloning DNA into
expression vectors, N23259

<400> 13
ggggcggccg cctatgctgc tggcaccagg ctag 34

<210> 14
<211> 29
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide for 3' RACE, N21965

<400> 14
tggtttcgtt accgacaacc ttgtatccc 29

<210> 15
<211> 28
<212> DNA
<213> Artificial Sequence

<220>
<223> Designed oligonucleotide for 5' race, N21968

<400> 15
gagttggtcc cagacagact tttgtcgt 28

<210> 16
<211> 1673
<212> DNA
<213> Exophiala spinifera

<220>
<221> sig_peptide
<222> (1)...(267)
<223> yeast alpha mating factor secretion signal.

<221> CDS
<222> (1)...(1662)

<400> 16
atg aga ttt cct tca att ttt act gct gtt tta ttc gca gca tcc tcc 48

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Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
                -85                 -80                 -75

gca tta gct gct cca gtc aac act aca aca gaa gat gaa acg gca caa       96
Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
            -70                 -65                 -60

att ccg gct gaa gct gtc atc ggt tac tca gat tta gaa ggg gat ttc      144
Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe
        -55                 -50                 -45

gat gtt gct gtt ttg cca ttt tcc aac agc aca aat aac ggg tta ttg      192
Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
    -40                 -35                 -30

ttt ata aat act act att gcc agc att gct gct aaa gaa gaa ggg gta      240
Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
-25                 -20                 -15                 -10

tct ctc gag aaa aga gag gct gaa gct gaa ttc aaa gac aac gtt gcg      288
Ser Leu Glu Lys Arg Glu Ala Glu Ala Glu Phe Lys Asp Asn Val Ala
                -5                  1               5

gac gtg gta gtg gtg ggc gct ggc ttg agc ggt ttg gag acg gca cgc      336
Asp Val Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg
        10                  15                  20

aaa gtc cag gcc gcc ggt ctg tcc tgc ctc gtt ctt gag gcg atg gat      384
Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp
    25                  30                  35

cgt gta ggg gga aag act ctg agc gta caa tcg ggt ccc ggc agg acg      432
Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr
40                  45                  50                  55

act atc aac gac ctc ggc gct gcg tgg atc aat gac agc aac caa agc      480
Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser
                60                  65                  70

gaa gta tcc aga ttg ttt gaa aga ttt cat ttg gag ggc gag ctc cag      528
Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln
            75                  80                  85

agg acg act gga aat tca atc cat caa gca caa gac ggt aca acc act      576
Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr
        90                  95                  100

aca gct cct tat ggt gac tcc ttg ctg agc gag gag gtt gca agt gca      624
Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala
    105                 110                 115

ctt gcg gaa ctc ctc ccc gta tgg tct cag ctg atc gaa gag cat agc      672
Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser
120                 125                 130                 135

ctt caa gac ctc aag gcg agc cct cag gcg aag cgg ctc gac agt gtg      720
Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val
                140                 145                 150

agc ttc gcg cac tac tgt gag aag gaa cta aac ttg cct gct gtt ctc      768
Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu
            155                 160                 165
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ggc gta gca aac cag atc aca cgc gct ctg ctc ggt gtg gaa gcc cac 816
Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His
170 175 180

gag atc agc atg ctt ttt ctc acc gac tac atc aag agt gcc acc ggt 864
Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly
185 190 195

ctc agt aat att ttc tcg gac aag aaa gac ggc ggg cag tat atg cga 912
Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg
200 205 210 215

tgc aaa aca ggt atg cag tcg att tgc cat gcc atg tca aag gaa ctt 960
Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu
220 225 230

gtt cca ggc tca gtg cac ctc aac acc ccc gtc gct gaa att gag cag 1008
Val Pro Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln
235 240 245

tcg gca tcc ggc tgt aca gta cga tcg gcc tcg ggc gcc gtg ttc cga 1056
Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg
250 255 260

agc aaa aag gtg gtg gtt tcg tta ccg aca acc ttg tat ccc acc ttg 1104
Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu
265 270 275

aca ttt tca cca cct ctt ccc gcc gag aag caa gca ttg gcg gaa aat 1152
Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn
280 285 290 295

tct atc ctg ggc tac tat agc aag ata gtc ttc gta tgg gac aag ccg 1200
Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro
300 305 310

tgg tgg cgc gaa caa ggc ttc tcg ggc gtc ctc caa tcg agc tgt gac 1248
Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp
315 320 325

ccc atc tca ttt gcc aga gat acc agc atc gac gtc gat cga caa tgg 1296
Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp
330 335 340

tcc att acc tgt ttc atg gtc gga gac ccg gga cgg aag tgg tcc caa 1344
Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln
345 350 355

cag tcc aag cag gta cga caa aag tct gtc tgg gac caa ctc cgc gca 1392
Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala
360 365 370 375

gcc tac gag aac gcc ggg gcc caa gtc cca gag ccg gcc aac gtg ctc 1440
Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu
380 385 390

gaa atc gag tgg tcg aag cag cag tat ttc caa gga gct ccg agc gcc 1488
Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala
395 400 405

gtc tat ggg ctg aac gat ctc atc aca ctg ggt tcg gcg ctc aga acg 1536

Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr
410 415 420

ccg ttc aag agt gtt cat ttc gtt gga acg gag acg tct tta gtt tgg 1584
Pro Phe Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp
425 430 435

aaa ggg tat atg gaa ggg gcc ata cga tcg ggt caa cga ggt gct gca 1632
Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala
440 445 450 455

gaa gtt gtg gct agc ctg gtg cca gca gca taggcggccg c 1673
Glu Val Val Ala Ser Leu Val Pro Ala Ala
460 465

<210> 17
<211> 554
<212> PRT
<213> Exophiala spinifera

<220>
<221> SIGNAL
<222> (1)...(89)

<400> 17
Met Arg Phe Pro Ser Ile Phe Thr Ala Val Leu Phe Ala Ala Ser Ser
-85 -80 -75
Ala Leu Ala Ala Pro Val Asn Thr Thr Thr Glu Asp Glu Thr Ala Gln
-70 -65 -60
Ile Pro Ala Glu Ala Val Ile Gly Tyr Ser Asp Leu Glu Gly Asp Phe
-55 -50 -45
Asp Val Ala Val Leu Pro Phe Ser Asn Ser Thr Asn Asn Gly Leu Leu
-40 -35 -30
Phe Ile Asn Thr Thr Ile Ala Ser Ile Ala Ala Lys Glu Glu Gly Val
-25 -20 -15 -10
Ser Leu Glu Lys Arg Glu Ala Glu Ala Glu Phe Lys Asp Asn Val Ala
-5 1 5
Asp Val Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg
10 15 20
Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp
25 30 35
Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr
40 45 50 55
Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser
60 65 70
Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln
75 80 85
Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr
90 95 100
Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala
105 110 115
Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser
120 125 130 135
Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val
140 145 150
Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu
155 160 165
Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His
170 175 180
Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly
185 190 195

Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg
200 205 210 215
Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu
220 225 230
Val Pro Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln
235 240 245
Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg
250 255 260
Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu
265 270 275
Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn
280 285 290 295
Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro
300 305 310
Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp
315 320 325
Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp
330 335 340
Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln
345 350 355
Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala
360 365 370 375
Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu
380 385 390
Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala
395 400 405
Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr
410 415 420
Pro Phe Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp
425 430 435
Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala
440 445 450 455
Glu Val Val Ala Ser Leu Val Pro Ala Ala
460 465

<210> 18
<211> 2079
<212> DNA
<213> Unknown

<220>
<223> GST:K:trAPAO 2079 nt. Translation starting at nt 1 - 687, gst fusion + polylinker, 688-2076, K:trAPAO, extra lysine underlined; 2077-2079, stop codon. For bacterial expression.

<221> CDS
<222> (1)...(2076)

<221> misc_feature
<222> (1)...(687)
<223> gst fusion + polylinker

<221> misc_feature
<222> (688)...(2076)
<223> K:trAPAO

<221> misc_feature
<222> (688)...(690)
<223> Extra lysine

<400> 18

atg tcc cct ata cta ggt tat tgg aaa att aag ggc ctt gtg caa ccc 48
Met Ser Pro Ile Leu Gly Tyr Trp Lys Ile Lys Gly Leu Val Gln Pro
1 5 10 15

act cga ctt ctt ttg gaa tat ctt gaa gaa aaa tat gaa gag cat ttg 96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
20 25 30

tat gag cgc gat gaa ggt gat aaa tgg cga aac aaa aag ttt gaa ttg 144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
35 40 45

ggt ttg gag ttt ccc aat ctt cct tat tat att gat ggt gat gtt aaa 192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
50 55 60

tta aca cag tct atg gcc atc ata cgt tat ata gct gac aag cac aac 240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65 70 75 80

atg ttg ggt ggt tgt cca aaa gag cgt gca gag att tca atg ctt gaa 288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
85 90 95

gga gcg gtt ttg gat att aga tac ggt gtt tcg aga att gca tat agt 336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
100 105 110

aaa gac ttt gaa act ctc aaa gtt gat ttt ctt agc aag cta cct gaa 384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
115 120 125

atg ctg aaa atg ttc gaa gat cgt tta tgt cat aaa aca tat tta aat 432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
130 135 140

ggt gat cat gta acc cat cct gac ttc atg ttg tat gac gct ctt gat 480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145 150 155 160

gtt gtt tta tac atg gac cca atg tgc ctg gat gcg ttc cca aaa tta 528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
165 170 175

gtt tgt ttt aaa aaa cgt att gaa gct atc cca caa att gat aag tac 576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
180 185 190

ttg aaa tcc agc aag tat ata gca tgg cct ttg cag ggc tgg caa gcc 624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
195 200 205

acg ttt ggt ggt ggc gac cat cct cca aaa tcg gat ctg gtt ccg cgt 672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
210 215 220

gga tcc ccg gaa ttc aaa gac aac gtt gcg gac gtg gta gtg gtg ggc 720
Gly Ser Pro Glu Phe Lys Asp Asn Val Ala Asp Val Val Val Val Gly
225 230 235 240

gct ggc ttg agc ggt ttg gag acg gca cgc aaa gtc cag gcc gcc ggt 768

Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly
245 250 255

ctg tcc tgc ctc gtt ctt gag gcg atg gat cgt gta ggg gga aag act 816
Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr
260 265 270

ctg agc gta caa tcg ggt ccc ggc agg acg act atc aac gac ctc ggc 864
Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly
275 280 285

gct gcg tgg atc aat gac agc aac caa agc gaa gta tcc aga ttg ttt 912
Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe
290 295 300

gaa aga ttt cat ttg gag ggc gag ctc cag agg acg act gga aat tca 960
Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser
305 310 315 320

atc cat caa gca caa gac ggt aca acc act aca gct cct tat ggt gac 1008
Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp
325 330 335

tcc ttg ctg agc gag gag gtt gca agt gca ctt gcg gaa ctc ctc ccc 1056
Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro
340 345 350

gta tgg tct cag ctg atc gaa gag cat agc ctt caa gac ctc aag gcg 1104
Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala
355 360 365

agc cct cag gcg aag cgg ctc gac agt gtg agc ttc gcg cac tac tgt 1152
Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys
370 375 380

gag aag gaa cta aac ttg cct gct gtt ctc ggc gta gca aac cag atc 1200
Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile
385 390 395 400

aca cgc gct ctg ctc ggt gtg gaa gcc cac gag atc agc atg ctt ttt 1248
Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser Met Leu Phe
405 410 415

ctc acc gac tac atc aag agt gcc acc ggt ctc agt aat att ttc tcg 1296
Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser
420 425 430

gac aag aaa gac ggc ggg cag tat atg cga tgc aaa aca ggt atg cag 1344
Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln
435 440 445

tcg att tgc cat gcc atg tca aag gaa ctt gtt cca ggc tca gtg cac 1392
Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro Gly Ser Val His
450 455 460

ctc aac acc ccc gtc gct gaa att gag cag tcg gca tcc ggc tgt aca 1440
Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr
465 470 475 480

gta cga tcg gcc tcg ggc gcc gtg ttc cga agc aaa aag gtg gtg gtt 1488
Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys Lys Val Val Val
485 490 495

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tcg tta ccg aca acc ttg tat ccc acc ttg aca ttt tca cca cct ctt      1536
Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu
            500                 505                 510

ccc gcc gag aag caa gca ttg gcg gaa aat tct atc ctg ggc tac tat      1584
Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr
        515                 520                 525

agc aag ata gtc ttc gta tgg gac aag ccg tgg tgg cgc gaa caa ggc      1632
Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly
    530                 535                 540

ttc tcg ggc gtc ctc caa tcg agc tgt gac ccc atc tca ttt gcc aga      1680
Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg
545                 550                 555                 560

gat acc agc atc gac gtc gat cga caa tgg tcc att acc tgt ttc atg      1728
Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met
                565                 570                 575

gtc gga gac ccg gga cgg aag tgg tcc caa cag tcc aag cag gta cga      1776
Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg
            580                 585                 590

caa aag tct gtc tgg gac caa ctc cgc gca gcc tac gag aac gcc ggg      1824
Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly
        595                 600                 605

gcc caa gtc cca gag ccg gcc aac gtg ctc gaa atc gag tgg tcg aag      1872
Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys
    610                 615                 620

cag cag tat ttc caa gga gct ccg agc gcc gtc tat ggg ctg aac gat      1920
Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp
625                 630                 635                 640

ctc atc aca ctg ggt tcg gcg ctc aga acg ccg ttc aag agt gtt cat      1968
Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His
                645                 650                 655

ttc gtt gga acg gag acg tct tta gtt tgg aaa ggg tat atg gaa ggg      2016
Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly
            660                 665                 670

gcc ata cga tcg ggt caa cga ggt gct gca gaa gtt gtg gct agc ctg      2064
Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu
        675                 680                 685

gtg cca gca gca tag                                                  2079
Val Pro Ala Ala
    690


   <210> 19
   <211> 692
   <212> PRT
   <213> Unknown

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 1               5                  10                  15
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20 25 30
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
35 40 45
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
50 55 60
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65 70 75 80
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
85 90 95
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
100 105 110
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
115 120 125
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
130 135 140
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145 150 155 160
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
165 170 175
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
180 185 190
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
195 200 205
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
210 215 220
Gly Ser Pro Glu Phe Lys Asp Asn Val Ala Asp Val Val Val Val Gly
225 230 235 240
Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly
245 250 255
Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr
260 265 270
Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly
275 280 285
Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe
290 295 300
Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser
305 310 315 320
Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp
325 330 335
Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro
340 345 350
Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala
355 360 365
Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys
370 375 380
Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile
385 390 395 400
Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser Met Leu Phe
405 410 415
Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser
420 425 430
Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln
435 440 445
Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro Gly Ser Val His
450 455 460
Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr
465 470 475 480
Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys Lys Val Val Val
485 490 495
Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu
500 505 510

Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr
        515   520   525
Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly
    530   535   540
Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg
545   550   555   560
Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met
                565   570   575
Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg
            580   585   590
Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly
        595   600   605
Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys
    610   615   620
Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp
625   630   635   640
Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His
                645   650   655
Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly
            660   665   670
Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu
        675   680   685
Val Pro Ala Ala
    690

<210> 20
<211> 1464
<212> DNA
<213> Unknown

<220>
<223> Nucleotide sequence of K:trAPAO translational fusion with barley alpha amylase signal sequence, for expression and secretion of the mature trAPAO in maize. Nucleotides 1-72, barley alpha amylase signal sequence, nucleotides 73-75, added lysine residue; nucleotides 76 -1464 , trAPAO cDNA.

<221> sig_peptide
<222> (1)...(72)
<223> Barley alpha amylase signal sequence

<221> misc_feature
<222> (73)...(1464)
<223> K:trAPAOcDNA

<221> CDS
<222> (1)...(1461)

<221> misc_feature
<222> (73)...(75)
<223> Added lysine residue

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Met Ala Asn Lys His Leu Ser Leu Ser Leu Phe Leu Val Leu Leu Gly
                -20   -15   -10

ctc tcc gcc tcc ctc gcc agc ggc aaa gac aac gtt gcg gac gtg gta 96
Leu Ser Ala Ser Leu Ala Ser Gly Lys Asp Asn Val Ala Asp Val Val
            -5   1   5

gtg gtg ggc gct ggc ttg agc ggt ttg gag acg gca cgc aaa gtc cag 144
Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln
10 15 20

gcc gcc ggt ctg tcc tgc ctc gtt ctt gag gcg atg gat cgt gta ggg 192
Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly
25 30 35 40

gga aag act ctg agc gta caa tcg ggt ccc ggc agg acg act atc aac 240
Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn
45 50 55

gac ctc ggc gct gcg tgg atc aat gac agc aac caa agc gaa gta tcc 288
Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Ser
60 65 70

aga ttg ttt gaa aga ttt cat ttg gag ggc gag ctc cag agg acg act 336
Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr
75 80 85

gga aat tca atc cat caa gca caa gac ggt aca acc act aca gct cct 384
Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro
90 95 100

tat ggt gac tcc ttg ctg agc gag gag gtt gca agt gca ctt gcg gaa 432
Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu
105 110 115 120

ctc ctc ccc gta tgg tct cag ctg atc gaa gag cat agc ctt caa gac 480
Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln Asp
125 130 135

ctc aag gcg agc cct cag gcg aag cgg ctc gac agt gtg agc ttc gcg 528
Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe Ala
140 145 150

cac tac tgt gag aag gaa cta aac ttg cct gct gtt ctc ggc gta gca 576
His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val Ala
155 160 165

aac cag atc aca cgc gct ctg ctc ggt gtg gaa gcc cac gag atc agc 624
Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser
170 175 180

atg ctt ttt ctc acc gac tac atc aag agt gcc acc ggt ctc agt aat 672
Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn
185 190 195 200

att ttc tcg gac aag aaa gac ggc ggg cag tat atg cga tgc aaa aca 720
Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr
205 210 215

ggt atg cag tcg att tgc cat gcc atg tca aag gaa ctt gtt cca ggc 768
Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro Gly
220 225 230

tca gtg cac ctc aac acc ccc gtc gct gaa att gag cag tcg gca tcc 816
Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser
235 240 245

ggc tgt aca gta cga tcg gcc tcg ggc gcc gtg ttc cga agc aaa aag 864

Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys Lys
250 255 260

gtg gtg gtt tcg tta ccg aca acc ttg tat ccc acc ttg aca ttt tca 912
Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe Ser
265 270 275 280

cca cct ctt ccc gcc gag aag caa gca ttg gcg gaa aat tct atc ctg 960
Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile Leu
285 290 295

ggc tac tat agc aag ata gtc ttc gta tgg gac aag ccg tgg tgg cgc 1008
Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg
300 305 310

gaa caa ggc ttc tcg ggc gtc ctc caa tcg agc tgt gac ccc atc tca 1056
Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser
315 320 325

ttt gcc aga gat acc agc atc gac gtc gat cga caa tgg tcc att acc 1104
Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile Thr
330 335 340

tgt ttc atg gtc gga gac ccg gga cgg aag tgg tcc caa cag tcc aag 1152
Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys
345 350 355 360

cag gta cga caa aag tct gtc tgg gac caa ctc cgc gca gcc tac gag 1200
Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr Glu
365 370 375

aac gcc ggg gcc caa gtc cca gag ccg gcc aac gtg ctc gaa atc gag 1248
Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile Glu
380 385 390

tgg tcg aag cag cag tat ttc caa gga gct ccg agc gcc gtc tat ggg 1296
Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr Gly
395 400 405

ctg aac gat ctc atc aca ctg ggt tcg gcg ctc aga acg ccg ttc aag 1344
Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe Lys
410 415 420

agt gtt cat ttc gtt gga acg gag acg tct tta gtt tgg aaa ggg tat 1392
Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr
425 430 435 440

atg gaa ggg gcc ata cga tcg ggt caa cga ggt gct gca gaa gtt gtg 1440
Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val Val
445 450 455

gct agc ctg gtg cca gca gca tag 1464
Ala Ser Leu Val Pro Ala Ala
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<213> Unknown

<220>
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<222> (1)...(24)

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Leu Ser Ala Ser Leu Ala Ser Gly Lys Asp Asn Val Ala Asp Val Val
            -5                  1               5
Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln
    10                  15                  20
Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly
25                  30                  35                  40
Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn
                45                  50                  55
Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Ser
            60                  65                  70
Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr
        75                  80                  85
Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro
    90                  95                  100
Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu
105                 110                 115                 120
Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln Asp
                125                 130                 135
Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe Ala
            140                 145                 150
His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val Ala
        155                 160                 165
Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser
    170                 175                 180
Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn
185                 190                 195                 200
Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr
                205                 210                 215
Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro Gly
            220                 225                 230
Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser
        235                 240                 245
Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys Lys
    250                 255                 260
Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe Ser
265                 270                 275                 280
Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile Leu
                285                 290                 295
Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg
            300                 305                 310
Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser
        315                 320                 325
Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile Thr
    330                 335                 340
Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys
345                 350                 355                 360
Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr Glu
                365                 370                 375
Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile Glu
            380                 385                 390
Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr Gly
        395                 400                 405
Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe Lys
    410                 415                 420
Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr
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425 430 435 440
Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val Val
445 450 455
Ala Ser Leu Val Pro Ala Ala
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<212> DNA
<213> Exophiala spinifera

<220>
<221> CDS
<222> (1)...(1800)

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Met Ala Leu Ala Pro Ser Tyr Ile Asn Pro Pro Asn Val Ala Ser Pro
1 5 10 15

gca ggg tat tct cac gtc ggc gta ggc cca gac gga ggg agg tat gtg 96
Ala Gly Tyr Ser His Val Gly Val Gly Pro Asp Gly Gly Arg Tyr Val
20 25 30

aca ata gct gga cag att gga caa gac gct tcg ggc gtg aca gac cct 144
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Gly Val Thr Asp Pro
35 40 45

gcc tac gag aaa cag gtt gcc caa gca ttc gcc aat ctg cga gct tgc 192
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
50 55 60

ctt gct gca gtt gga gcc act tca aac gac gtc acc aag ctc aat tac 240
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Val Thr Lys Leu Asn Tyr
65 70 75 80

tac atc gtc gac tac gcc ccg agc aaa ctc acc gca att gga gat ggg 288
Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
85 90 95

ctg aag gct acc ttt gcc ctt gac agg ctc cct cct tgc acg ctg gtg 336
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
100 105 110

cca gtg tcg gcc ttg tct tca cct gaa tac ctc ttt gag gtt gat gcc 384
Pro Val Ser Ala Leu Ser Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
115 120 125

acg gcg ctg gtg ccg gga cac acg acc cca gac aac gtt gcg gac gtg 432
Thr Ala Leu Val Pro Gly His Thr Thr Pro Asp Asn Val Ala Asp Val
130 135 140

gta gtg gtg ggc gct ggc ttg agc ggt ttg gag acg gca cgc aaa gtc 480
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145 150 155 160

cag gcc gcc ggt ctg tcc tgc ctc gtt ctt gag gcg atg gat cgt gta 528
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
165 170 175

ggg gga aag act ctg agc gta caa tcg ggt ccc ggc agg acg act atc 576
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile

180 185 190

aac gac ctc ggc gct gcg tgg atc aat gac agc aac caa agc gaa gta 624
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
195 200 205

tcc aga ttg ttt gaa aga ttt cat ttg gag ggc gag ctc cag agg acg 672
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
210 215 220

act gga aat tca atc cat caa gca caa gac ggt aca acc act aca gct 720
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
225 230 235 240

cct tat ggt gac tcc ttg ctg agc gag gag gtt gca agt gca ctt gcg 768
Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
245 250 255

gaa ctc ctc ccc gta tgg tct cag ctg atc gaa gag cat agc ctt caa 816
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln
260 265 270

gac ctc aag gcg agc cct cag gcg aag cgg ctc gac agt gtg agc ttc 864
Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
275 280 285

gcg cac tac tgt gag aag gaa cta aac ttg cct gct gtt ctc ggc gta 912
Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val
290 295 300

gca aac cag atc aca cgc gct ctg ctc ggt gtg gaa gcc cac gag atc 960
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
305 310 315 320

agc atg ctt ttt ctc acc gac tac atc aag agt gcc acc ggt ctc agt 1008
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
325 330 335

aat att ttc tcg gac aag aaa gac ggc ggg cag tat atg cga tgc aaa 1056
Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
340 345 350

aca ggt atg cag tcg att tgc cat gcc atg tca aag gaa ctt gtt cca 1104
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
355 360 365

ggc tca gtg cac ctc aac acc ccc gtc gct gaa att gag cag tcg gca 1152
Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala
370 375 380

tcc ggc tgt aca gta cga tcg gcc tcg ggc gcc gtg ttc cga agc aaa 1200
Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
385 390 395 400

aag gtg gtg gtt tcg tta ccg aca acc ttg tat ccc acc ttg aca ttt 1248
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
405 410 415

tca cca cct ctt ccc gcc gag aag caa gca ttg gcg gaa aat tct atc 1296
Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile
420 425 430

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ctg ggc tac tat agc aag ata gtc ttc gta tgg gac aag ccg tgg tgg      1344
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp
        435                 440                 445

cgc gaa caa ggc ttc tcg ggc gtc ctc caa tcg agc tgt gac ccc atc      1392
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
    450                 455                 460

tca ttt gcc aga gat acc agc atc gac gtc gat cga caa tgg tcc att      1440
Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile
465                 470                 475                 480

acc tgt ttc atg gtc gga gac ccg gga cgg aag tgg tcc caa cag tcc      1488
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
                485                 490                 495

aag cag gta cga caa aag tct gtc tgg gac caa ctc cgc gca gcc tac      1536
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
            500                 505                 510

gag aac gcc ggg gcc caa gtc cca gag ccg gcc aac gtg ctc gaa atc      1584
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
        515                 520                 525

gag tgg tcg aag cag cag tat ttc caa gga gct ccg agc gcc gtc tat      1632
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
    530                 535                 540

ggg ctg aac gat ctc atc aca ctg ggt tcg gcg ctc aga acg ccg ttc      1680
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
545                 550                 555                 560

aag agt gtt cat ttc gtt gga acg gag acg tct tta gtt tgg aaa ggg      1728
Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
                565                 570                 575

tat atg gaa ggg gcc ata cga tcg ggt caa cga ggt gct gca gaa gtt      1776
Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val
            580                 585                 590

gtg gct agc ctg gtg cca gca gca tag                                  1803
Val Ala Ser Leu Val Pro Ala Ala
        595                 600


<210> 23
<211> 600
<212> PRT
<213> Exophiala spinifera

<400> 23
Met Ala Leu Ala Pro Ser Tyr Ile Asn Pro Pro Asn Val Ala Ser Pro
1               5                   10                  15
Ala Gly Tyr Ser His Val Gly Val Gly Pro Asp Gly Gly Arg Tyr Val
            20                  25                  30
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Gly Val Thr Asp Pro
        35                  40                  45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
    50                  55                  60
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Val Thr Lys Leu Asn Tyr
65                  70                  75                  80
Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
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85 90 95
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
100 105 110
Pro Val Ser Ala Leu Ser Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
115 120 125
Thr Ala Leu Val Pro Gly His Thr Thr Pro Asp Asn Val Ala Asp Val
130 135 140
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145 150 155 160
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
165 170 175
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
180 185 190
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
195 200 205
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
210 215 220
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
225 230 235 240
Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
245 250 255
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln
260 265 270
Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
275 280 285
Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val
290 295 300
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
305 310 315 320
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
325 330 335
Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
340 345 350
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
355 360 365
Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala
370 375 380
Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
385 390 395 400
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
405 410 415
Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile
420 425 430
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp
435 440 445
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
450 455 460
Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile
465 470 475 480
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
485 490 495
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
500 505 510
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
515 520 525
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
530 535 540
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
545 550 555 560
Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
565 570 575
Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val

580 585 590
Val Ala Ser Leu Val Pro Ala Ala
595 600

<210> 24
<211> 3003
<212> DNA
<213> Unknown

<220>
<223> Sequence is barley alpha amylase signal sequence:
esp1 mat: an artificial spacer sequence and
K:trAPAO

<221> sig_peptide
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<223> Barley alpha amylase signal sequence

<221> misc_feature
<222> (73)...(1575)
<223> esp1 mat

<221> misc_feature
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<223> spacer sequence

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<223> K:trAPAO

<221> CDS
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<222> (1612)...(1614)
<223> Extra lysine

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Met Ala Asn Lys His Leu Ser Leu Ser Leu Phe Leu Val Leu Leu Gly
-20 -15 -10

ctc tcc gcc tcc ctc gcc agc ggc gct cct act gtc aag att gat gct 96
Leu Ser Ala Ser Leu Ala Ser Gly Ala Pro Thr Val Lys Ile Asp Ala
-5 1 5

ggg atg gtg gtc ggc acg act act act gtc ccc ggc acc act gcg acc 144
Gly Met Val Val Gly Thr Thr Thr Thr Val Pro Gly Thr Thr Ala Thr
10 15 20

gtc agc gag ttc ttg ggc gtt cct ttt gcc gcc tct ccg aca cga ttt 192
Val Ser Glu Phe Leu Gly Val Pro Phe Ala Ala Ser Pro Thr Arg Phe
25 30 35 40

gcg cct cct act cgt ccc gtg cct tgg tca acg cct ttg caa gcc act 240
Ala Pro Pro Thr Arg Pro Val Pro Trp Ser Thr Pro Leu Gln Ala Thr
45 50 55

gca tat ggt cca gca tgc cct caa caa ttc aat tac ccc gaa gaa ctc 288
Ala Tyr Gly Pro Ala Cys Pro Gln Gln Phe Asn Tyr Pro Glu Glu Leu
60 65 70

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cgt gag att acg atg gcc tgg ttc aat aca ccg ccc ccg tca gct ggt      336
Arg Glu Ile Thr Met Ala Trp Phe Asn Thr Pro Pro Pro Ser Ala Gly
         75                  80                  85

gaa agt gag gac tgc ctg aac ctc aac atc tac gtc cca gga act gag      384
Glu Ser Glu Asp Cys Leu Asn Leu Asn Ile Tyr Val Pro Gly Thr Glu
     90                  95                  100

aac aca aac aaa gcc gtc atg gtt tgg ata tac ggt gga gcg ctg gaa      432
Asn Thr Asn Lys Ala Val Met Val Trp Ile Tyr Gly Gly Ala Leu Glu
105                 110                 115                 120

tat ggt tgg aat tca ttc cac ctt tac gac ggg gct agt ttc gca gcc      480
Tyr Gly Trp Asn Ser Phe His Leu Tyr Asp Gly Ala Ser Phe Ala Ala
                125                 130                 135

aat cag gat gtc atc gcc gtg acc atc aac tac aga acg aac att ctg      528
Asn Gln Asp Val Ile Ala Val Thr Ile Asn Tyr Arg Thr Asn Ile Leu
            140                 145                 150

ggg ttc cct gct gcc cct cag ctt cca ata aca cag cga aat ctg ggg      576
Gly Phe Pro Ala Ala Pro Gln Leu Pro Ile Thr Gln Arg Asn Leu Gly
        155                 160                 165

ttc cta gac caa agg ttt gct ttg gat tgg gta cag cgg aac atc gca      624
Phe Leu Asp Gln Arg Phe Ala Leu Asp Trp Val Gln Arg Asn Ile Ala
    170                 175                 180

gcc ttt ggc ggt gat cct cga aag gtc aca ata ttt ggg cag agt gcg      672
Ala Phe Gly Gly Asp Pro Arg Lys Val Thr Ile Phe Gly Gln Ser Ala
185                 190                 195                 200

ggg ggc aga agt gtc gac gtc ctc ttg acg tct atg cca cac aac cca      720
Gly Gly Arg Ser Val Asp Val Leu Leu Thr Ser Met Pro His Asn Pro
                205                 210                 215

ccc ttc cga gca gca atc atg gag tcc ggt gtg gct aac tac aac ttc      768
Pro Phe Arg Ala Ala Ile Met Glu Ser Gly Val Ala Asn Tyr Asn Phe
            220                 225                 230

ccc aag gga gat ttg tcc gaa cct tgg aac acc act gtt caa gct ctc      816
Pro Lys Gly Asp Leu Ser Glu Pro Trp Asn Thr Thr Val Gln Ala Leu
        235                 240                 245

aac tgt acc acc agt atc gac atc ttg agt tgt atg aga aga gtc gat      864
Asn Cys Thr Thr Ser Ile Asp Ile Leu Ser Cys Met Arg Arg Val Asp
    250                 255                 260

ctc gcc act ctg atg aac acg atc gag caa ctc gga ctt ggg ttt gag      912
Leu Ala Thr Leu Met Asn Thr Ile Glu Gln Leu Gly Leu Gly Phe Glu
265                 270                 275                 280

tac acg ttg gac aac gta acg gct gtg tac cgt tct gaa acg gct cgc      960
Tyr Thr Leu Asp Asn Val Thr Ala Val Tyr Arg Ser Glu Thr Ala Arg
                285                 290                 295

acg act ggt gac att gct cgt gta cct gtt ctc gtc ggg acg gtg gcc     1008
Thr Thr Gly Asp Ile Ala Arg Val Pro Val Leu Val Gly Thr Val Ala
            300                 305                 310

aac gac gga ctt ctc ttt gtc ctc ggg gag aat gac acc caa gca tat     1056
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Asn Asp Gly Leu Leu Phe Val Leu Gly Glu Asn Asp Thr Gln Ala Tyr
        315                 320                 325

ctc gag gag gca atc ccg aat cag ccc gac ctt tac cag act ctc ctt      1104
Leu Glu Glu Ala Ile Pro Asn Gln Pro Asp Leu Tyr Gln Thr Leu Leu
    330                 335                 340

gga gca tat ccc att gga tcc cca ggg atc gga tcg cct caa gat cag      1152
Gly Ala Tyr Pro Ile Gly Ser Pro Gly Ile Gly Ser Pro Gln Asp Gln
345                 350                 355                 360

att gcc gcc att gag acc gag gta aga ttc cag tgt cct tct gcc atc      1200
Ile Ala Ala Ile Glu Thr Glu Val Arg Phe Gln Cys Pro Ser Ala Ile
                365                 370                 375

gtg gct cag gac tcc cgg aat cgg ggt atc cct tct tgg cgc tac tac      1248
Val Ala Gln Asp Ser Arg Asn Arg Gly Ile Pro Ser Trp Arg Tyr Tyr
            380                 385                 390

tac aat gcg acc ttt gag aat ctg gag ctt ttc cct ggg tcc gaa gtg      1296
Tyr Asn Ala Thr Phe Glu Asn Leu Glu Leu Phe Pro Gly Ser Glu Val
        395                 400                 405

tac cac agc tct gaa gtc ggg atg gtg ttt ggc acg tat cct gtc gca      1344
Tyr His Ser Ser Glu Val Gly Met Val Phe Gly Thr Tyr Pro Val Ala
    410                 415                 420

agt gcg acc gcc ttg gag gcc cag acg agc aaa tac atg cag ggt gcc      1392
Ser Ala Thr Ala Leu Glu Ala Gln Thr Ser Lys Tyr Met Gln Gly Ala
425                 430                 435                 440

tgg gcg gcc ttt gcc aaa aac ccc atg aat ggg cct ggg tgg aaa caa      1440
Trp Ala Ala Phe Ala Lys Asn Pro Met Asn Gly Pro Gly Trp Lys Gln
                445                 450                 455

gtg ccg aat gtc gcg gcg ctt ggc tca cca ggc aaa gcc atc cag gtt      1488
Val Pro Asn Val Ala Ala Leu Gly Ser Pro Gly Lys Ala Ile Gln Val
            460                 465                 470

gac gtc tct cca gcg aca ata gac caa cga tgt gcc ttg tac acg cgt      1536
Asp Val Ser Pro Ala Thr Ile Asp Gln Arg Cys Ala Leu Tyr Thr Arg
        475                 480                 485

tat tat act gag ttg ggc aca atc gcg ccg agg aca ttt ggc gga ggc      1584
Tyr Tyr Thr Glu Leu Gly Thr Ile Ala Pro Arg Thr Phe Gly Gly Gly
    490                 495                 500

agc ggc gga ggc agc ggc gga ggc agc aaa gac aac gtt gcg gac gtg      1632
Ser Gly Gly Gly Ser Gly Gly Gly Ser Lys Asp Asn Val Ala Asp Val
505                 510                 515                 520

gta gtg gtg ggc gct ggc ttg agc ggt ttg gag acg gca cgc aaa gtc      1680
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
                525                 530                 535

cag gcc gcc ggt ctg tcc tgc ctc gtt ctt gag gcg atg gat cgt gta      1728
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
            540                 545                 550

ggg gga aag act ctg agc gta caa tcg ggt ccc ggc agg acg act atc      1776
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
        555                 560                 565
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aac gac ctc ggc gct gcg tgg atc aat gac agc aac caa agc gaa gta      1824
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
    570                 575                 580

tcc aga ttg ttt gaa aga ttt cat ttg gag ggc gag ctc cag agg acg      1872
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
585                 590                 595                 600

act gga aat tca atc cat caa gca caa gac ggt aca acc act aca gct      1920
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
                605                 610                 615

cct tat ggt gac tcc ttg ctg agc gag gag gtt gca agt gca ctt gcg      1968
Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
            620                 625                 630

gaa ctc ctc ccc gta tgg tct cag ctg atc gaa gag cat agc ctt caa      2016
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln
        635                 640                 645

gac ctc aag gcg agc cct cag gcg aag cgg ctc gac agt gtg agc ttc      2064
Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
    650                 655                 660

gcg cac tac tgt gag aag gaa cta aac ttg cct gct gtt ctc ggc gta      2112
Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val
665                 670                 675                 680

gca aac cag atc aca cgc gct ctg ctc ggt gtg gaa gcc cac gag atc      2160
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
                685                 690                 695

agc atg ctt ttt ctc acc gac tac atc aag agt gcc acc ggt ctc agt      2208
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
            700                 705                 710

aat att ttc tcg gac aag aaa gac ggc ggg cag tat atg cga tgc aaa      2256
Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
        715                 720                 725

aca ggt atg cag tcg att tgc cat gcc atg tca aag gaa ctt gtt cca      2304
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
    730                 735                 740

ggc tca gtg cac ctc aac acc ccc gtc gct gaa att gag cag tcg gca      2352
Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala
745                 750                 755                 760

tcc ggc tgt aca gta cga tcg gcc tcg ggc gcc gtg ttc cga agc aaa      2400
Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
                765                 770                 775

aag gtg gtg gtt tcg tta ccg aca acc ttg tat ccc acc ttg aca ttt      2448
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
            780                 785                 790

tca cca cct ctt ccc gcc gag aag caa gca ttg gcg gaa aat tct atc      2496
Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile

        795                 800                 805
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ctg ggc tac tat agc aag ata gtc ttc gta tgg gac aag ccg tgg tgg 2544
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp
810 815 820

cgc gaa caa ggc ttc tcg ggc gtc ctc caa tcg agc tgt gac ccc atc 2592
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
825 830 835 840

tca ttt gcc aga gat acc agc atc gac gtc gat cga caa tgg tcc att 2640
Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile
845 850 855

acc tgt ttc atg gtc gga gac ccg gga cgg aag tgg tcc caa cag tcc 2688
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
860 865 870

aag cag gta cga caa aag tct gtc tgg gac caa ctc cgc gca gcc tac 2736
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
875 880 885

gag aac gcc ggg gcc caa gtc cca gag ccg gcc aac gtg ctc gaa atc 2784
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
890 895 900

gag tgg tcg aag cag cag tat ttc caa gga gct ccg agc gcc gtc tat 2832
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
905 910 915 920

ggg ctg aac gat ctc atc aca ctg ggt tcg gcg ctc aga acg ccg ttc 2880
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
925 930 935

aag agt gtt cat ttc gtt gga acg gag acg tct tta gtt tgg aaa ggg 2928
Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
940 945 950

tat atg gaa ggg gcc ata cga tcg ggt caa cga ggt gct gca gaa gtt 2976
Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val
955 960 965

gtg gct agc ctg gtg cca gca gca tag 3003
Val Ala Ser Leu Val Pro Ala Ala
970 975

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<211> 1000
<212> PRT
<213> Unknown

<220>
<221> SIGNAL
<222> (1)...(24)

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-20 -15 -10
Leu Ser Ala Ser Leu Ala Ser Gly Ala Pro Thr Val Lys Ile Asp Ala
-5 1 5
Gly Met Val Val Gly Thr Thr Thr Thr Val Pro Gly Thr Thr Ala Thr
10 15 20
Val Ser Glu Phe Leu Gly Val Pro Phe Ala Ala Ser Pro Thr Arg Phe

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25                  30                  35                  40
Ala Pro Pro Thr Arg Pro Val Pro Trp Ser Thr Pro Leu Gln Ala Thr
                45                  50                  55
Ala Tyr Gly Pro Ala Cys Pro Gln Gln Phe Asn Tyr Pro Glu Glu Leu
            60                  65                  70
Arg Glu Ile Thr Met Ala Trp Phe Asn Thr Pro Pro Pro Ser Ala Gly
        75                  80                  85
Glu Ser Glu Asp Cys Leu Asn Leu Asn Ile Tyr Val Pro Gly Thr Glu
    90                  95                  100
Asn Thr Asn Lys Ala Val Met Val Trp Ile Tyr Gly Gly Ala Leu Glu
105                 110                 115                 120
Tyr Gly Trp Asn Ser Phe His Leu Tyr Asp Gly Ala Ser Phe Ala Ala
                125                 130                 135
Asn Gln Asp Val Ile Ala Val Thr Ile Asn Tyr Arg Thr Asn Ile Leu
            140                 145                 150
Gly Phe Pro Ala Ala Pro Gln Leu Pro Ile Thr Gln Arg Asn Leu Gly
        155                 160                 165
Phe Leu Asp Gln Arg Phe Ala Leu Asp Trp Val Gln Arg Asn Ile Ala
    170                 175                 180
Ala Phe Gly Gly Asp Pro Arg Lys Val Thr Ile Phe Gly Gln Ser Ala
185                 190                 195                 200
Gly Gly Arg Ser Val Asp Val Leu Leu Thr Ser Met Pro His Asn Pro
                205                 210                 215
Pro Phe Arg Ala Ala Ile Met Glu Ser Gly Val Ala Asn Tyr Asn Phe
            220                 225                 230
Pro Lys Gly Asp Leu Ser Glu Pro Trp Asn Thr Thr Val Gln Ala Leu
        235                 240                 245
Asn Cys Thr Thr Ser Ile Asp Ile Leu Ser Cys Met Arg Arg Val Asp
    250                 255                 260
Leu Ala Thr Leu Met Asn Thr Ile Glu Gln Leu Gly Leu Gly Phe Glu
265                 270                 275                 280
Tyr Thr Leu Asp Asn Val Thr Ala Val Tyr Arg Ser Glu Thr Ala Arg
                285                 290                 295
Thr Thr Gly Asp Ile Ala Arg Val Pro Val Leu Val Gly Thr Val Ala
            300                 305                 310
Asn Asp Gly Leu Leu Phe Val Leu Gly Glu Asn Asp Thr Gln Ala Tyr
        315                 320                 325
Leu Glu Glu Ala Ile Pro Asn Gln Pro Asp Leu Tyr Gln Thr Leu Leu
    330                 335                 340
Gly Ala Tyr Pro Ile Gly Ser Pro Gly Ile Gly Ser Pro Gln Asp Gln
345                 350                 355                 360
Ile Ala Ala Ile Glu Thr Glu Val Arg Phe Gln Cys Pro Ser Ala Ile
                365                 370                 375
Val Ala Gln Asp Ser Arg Asn Arg Gly Ile Pro Ser Trp Arg Tyr Tyr
            380                 385                 390
Tyr Asn Ala Thr Phe Glu Asn Leu Glu Leu Phe Pro Gly Ser Glu Val
        395                 400                 405
Tyr His Ser Ser Glu Val Gly Met Val Phe Gly Thr Tyr Pro Val Ala
    410                 415                 420
Ser Ala Thr Ala Leu Glu Ala Gln Thr Ser Lys Tyr Met Gln Gly Ala
425                 430                 435                 440
Trp Ala Ala Phe Ala Lys Asn Pro Met Asn Gly Pro Gly Trp Lys Gln
                445                 450                 455
Val Pro Asn Val Ala Ala Leu Gly Ser Pro Gly Lys Ala Ile Gln Val
            460                 465                 470
Asp Val Ser Pro Ala Thr Ile Asp Gln Arg Cys Ala Leu Tyr Thr Arg
        475                 480                 485
Tyr Tyr Thr Glu Leu Gly Thr Ile Ala Pro Arg Thr Phe Gly Gly Gly
    490                 495                 500
Ser Gly Gly Gly Ser Gly Gly Gly Ser Lys Asp Asn Val Ala Asp Val
505                 510                 515                 520
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
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525 530 535
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
540 545 550
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
555 560 565
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
570 575 580
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
585 590 595 600
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
605 610 615
Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
620 625 630
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln
635 640 645
Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
650 655 660
Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val
665 670 675 680
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
685 690 695
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
700 705 710
Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
715 720 725
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
730 735 740
Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala
745 750 755 760
Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
765 770 775
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
780 785 790
Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile
795 800 805
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp
810 815 820
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
825 830 835 840
Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile
845 850 855
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
860 865 870
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
875 880 885
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
890 895 900
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
905 910 915 920
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
925 930 935
Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
940 945 950
Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val
955 960 965
Val Ala Ser Leu Val Pro Ala Ala
970 975

<210> 26
<211> 2976
<212> DNA
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<220>
<223> Barley alpha amylase signal sequence: BEST1
mature: artificial spacer: and K:trAPAO. For
plant expression.

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<223> Barley alpha amylase signal sequence

<221> mat_peptide
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<221> misc_feature
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<221> CDS
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<221> misc_feature
<222> (1585)...(1587)
<223> Extra lysine

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Met Ala Asn Lys His Leu Ser Leu Ser Leu Phe Leu Val Leu Leu Gly
-20 -15 -10

ctc tcc gcc tcc ctc gcc agc ggc acg gat ttt ccg gtc cgc agg acc 96
Leu Ser Ala Ser Leu Ala Ser Gly Thr Asp Phe Pro Val Arg Arg Thr
-5 1 5

gat ctg ggc cag gtt cag gga ctg gcc ggg gac gtg atg agc ttt cgc 144
Asp Leu Gly Gln Val Gln Gly Leu Ala Gly Asp Val Met Ser Phe Arg
10 15 20

gga ata ccc tat gca gcg ccg ccg gtg ggc ggg ctg cgt tgg aag ccg 192
Gly Ile Pro Tyr Ala Ala Pro Pro Val Gly Gly Leu Arg Trp Lys Pro
25 30 35 40

ccc caa cac gcc cgg ccc tgg gcg ggc gtt cgc ccc gcc acc caa ttt 240
Pro Gln His Ala Arg Pro Trp Ala Gly Val Arg Pro Ala Thr Gln Phe
45 50 55

ggc tcc gac tgc ttc ggc gcg gcc tat ctt cgc aaa ggc agc ctc gcc 288
Gly Ser Asp Cys Phe Gly Ala Ala Tyr Leu Arg Lys Gly Ser Leu Ala
60 65 70

ccc ggc gtg agc gag gac tgt ctt tac ctc aac gta tgg gcg ccg tca 336
Pro Gly Val Ser Glu Asp Cys Leu Tyr Leu Asn Val Trp Ala Pro Ser

75 80 85

ggc gct aaa ccc ggc cag tac ccc gtc atg gtc tgg gtc tac ggc ggc 384
Gly Ala Lys Pro Gly Gln Tyr Pro Val Met Val Trp Val Tyr Gly Gly

90 95 100

ggc ttc gcc ggc ggc acg gcc gcc atg ccc tac tac gac ggc gag gcg 432
Gly Phe Ala Gly Gly Thr Ala Ala Met Pro Tyr Tyr Asp Gly Glu Ala
105 110 115 120

ctt gcg cga cag ggc gtc gtc gtg gtg acg ttt aac tat cgg acg aac 480
Leu Ala Arg Gln Gly Val Val Val Val Thr Phe Asn Tyr Arg Thr Asn
125 130 135

atc ctg ggc ttt ttc gcc cat cct ggt ctc tcg cgc gag agc ccc acc 528
Ile Leu Gly Phe Phe Ala His Pro Gly Leu Ser Arg Glu Ser Pro Thr
140 145 150

gga act tcg ggc aac tac ggc cta ctc gac att ctc gcc gct ctt cgg 576
Gly Thr Ser Gly Asn Tyr Gly Leu Leu Asp Ile Leu Ala Ala Leu Arg
155 160 165

tgg gtg cag agc aac gcc cgc gcc ttc gga ggg gac ccc ggc cga gtg 624
Trp Val Gln Ser Asn Ala Arg Ala Phe Gly Gly Asp Pro Gly Arg Val
170 175 180

acg gtc ttt ggt gaa tcg gcc gga gcg agc gcg atc gga ctt ctg ctc 672
Thr Val Phe Gly Glu Ser Ala Gly Ala Ser Ala Ile Gly Leu Leu Leu
185 190 195 200

acc tcg ccg ctg agc aag ggt ctc ttc cgt ggc gct atc ctc gaa agt 720
Thr Ser Pro Leu Ser Lys Gly Leu Phe Arg Gly Ala Ile Leu Glu Ser
205 210 215

cca ggg ctg acg cga ccg ctc gcg acg ctc gcc gac agc gcc gcc tcg 768
Pro Gly Leu Thr Arg Pro Leu Ala Thr Leu Ala Asp Ser Ala Ala Ser
220 225 230

ggc gag cgc ctc gac gcc gat ctt tcg cga ctg cgc tcg acc gac cca 816
Gly Glu Arg Leu Asp Ala Asp Leu Ser Arg Leu Arg Ser Thr Asp Pro
235 240 245

gcc acc ctg atg gcg cgc gcc gac gcg gcc cgc ccg gca tcg cgg gac 864
Ala Thr Leu Met Ala Arg Ala Asp Ala Ala Arg Pro Ala Ser Arg Asp
250 255 260

ctg cgc agg ccg cgt ccg acc gga ccg atc gtc gat ggc cat gtg ctg 912
Leu Arg Arg Pro Arg Pro Thr Gly Pro Ile Val Asp Gly His Val Leu
265 270 275 280

ccg cag acc gac agc gcg gcg atc gcg gcg ggg cag ctg gcg ccg gtt 960
Pro Gln Thr Asp Ser Ala Ala Ile Ala Ala Gly Gln Leu Ala Pro Val
285 290 295

cgg gtc ctg atc gga acc aat gcc gac gaa ggc cgc gcc ttc ctc ggg 1008
Arg Val Leu Ile Gly Thr Asn Ala Asp Glu Gly Arg Ala Phe Leu Gly
300 305 310

cgc gcg ccg atg gag acg cca gcg gac tac caa gcc tat ctg gag gcg 1056
Arg Ala Pro Met Glu Thr Pro Ala Asp Tyr Gln Ala Tyr Leu Glu Ala
315 320 325

cag ttt ggc gac caa gcc gcc gcc gtg gcg gcg tgc tat ccc ctc gac 1104
Gln Phe Gly Asp Gln Ala Ala Ala Val Ala Ala Cys Tyr Pro Leu Asp
330 335 340

ggc cgg gcc acg ccc aag gaa atg gtc gcg cgc atc ttc ggc gac aat 1152

Gly Arg Ala Thr Pro Lys Glu Met Val Ala Arg Ile Phe Gly Asp Asn

345 350 355 360

cag ttc aat cgg ggg gtc tcg gcc ttc tcg gaa gcg ctt gtg cgc cag 1200

Gln Phe Asn Arg Gly Val Ser Ala Phe Ser Glu Ala Leu Val Arg Gln

365 370 375

ggc gcg ccc gtg tgg cgt tat cag ttc aac ggt aat acc gag ggt gga 1248

Gly Ala Pro Val Trp Arg Tyr Gln Phe Asn Gly Asn Thr Glu Gly Gly

380 385 390

aga gcg ccg gct acc cac gga gcc gaa att ccc tac gtt ttc ggg gtg 1296

Arg Ala Pro Ala Thr His Gly Ala Glu Ile Pro Tyr Val Phe Gly Val

395 400 405

ttc aag ctc gac gag ttg ggt ctg ttc gat tgg ccg ccc gag ggg ccc 1344

Phe Lys Leu Asp Glu Leu Gly Leu Phe Asp Trp Pro Pro Glu Gly Pro

410 415 420

acg ccc gcc gac cgt gcg ctg ggc caa ctg atg tcc tcc gcc tgg gtc 1392

Thr Pro Ala Asp Arg Ala Leu Gly Gln Leu Met Ser Ser Ala Trp Val

425 430 435 440

cgg ttc gcc aag aat ggc gac ccc gcc ggg gac gcc ctt acc tgg cct 1440

Arg Phe Ala Lys Asn Gly Asp Pro Ala Gly Asp Ala Leu Thr Trp Pro

445 450 455

gcc tat tct acg ggc aag tcg acc atg aca ttc ggt ccc gag ggc cgc 1488

Ala Tyr Ser Thr Gly Lys Ser Thr Met Thr Phe Gly Pro Glu Gly Arg

460 465 470

gcg gcg gtg gtg tcg ccc gga cct tcc atc ccc cct tgc gcg gat ggc 1536

Ala Ala Val Val Ser Pro Gly Pro Ser Ile Pro Pro Cys Ala Asp Gly

475 480 485

gcc aag gcg ggg ggc gga ggc agc ggc gga ggc agc ggc gga ggc agc 1584

Ala Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser

490 495 500

aaa gac aac gtt gcg gac gtg gta gtg gtg ggc gct ggc ttg agc ggt 1632

Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly

505 510 515 520

ttg gag acg gca cgc aaa gtc cag gcc gcc ggt ctg tcc tgc ctc gtt 1680

Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val

525 530 535

ctt gag gcg atg gat cgt gta ggg gga aag act ctg agc gta caa tcg 1728

Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser

540 545 550

ggt ccc ggc agg acg act atc aac gac ctc ggc gct gcg tgg atc aat 1776

Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn

555 560 565

gac agc aac caa agc gaa gta tcc aga ttg ttt gaa aga ttt cat ttg 1824

Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu

570 575 580

gag ggc gag ctc cag agg acg act gga aat tca atc cat caa gca caa 1872

Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln

585 590 595 600

gac ggt aca acc act aca gct cct tat ggt gac tcc ttg ctg agc gag 1920
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
605 610 615

gag gtt gca agt gca ctt gcg gaa ctc ctc ccc gta tgg tct cag ctg 1968
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
620 625 630

atc gaa gag cat agc ctt caa gac ctc aag gcg agc cct cag gcg aag 2016
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
635 640 645

cgg ctc gac agt gtg agc ttc gcg cac tac tgt gag aag gaa cta aac 2064
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
650 655 660

ttg cct gct gtt ctc ggc gta gca aac cag atc aca cgc gct ctg ctc 2112
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
665 670 675 680

ggt gtg gaa gcc cac gag atc agc atg ctt ttt ctc acc gac tac atc 2160
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
685 690 695

aag agt gcc acc ggt ctc agt aat att ttc tcg gac aag aaa gac ggc 2208
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
700 705 710

ggg cag tat atg cga tgc aaa aca ggt atg cag tcg att tgc cat gcc 2256
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala
715 720 725

atg tca aag gaa ctt gtt cca ggc tca gtg cac ctc aac acc ccc gtc 2304
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
730 735 740

gct gaa att gag cag tcg gca tcc ggc tgt aca gta cga tcg gcc tcg 2352
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
745 750 755 760

ggc gcc gtg ttc cga agc aaa aag gtg gtg gtt tcg tta ccg aca acc 2400
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
765 770 775

ttg tat ccc acc ttg aca ttt tca cca cct ctt ccc gcc gag aag caa 2448
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
780 785 790

gca ttg gcg gaa aat tct atc ctg ggc tac tat agc aag ata gtc ttc 2496
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
795 800 805

gta tgg gac aag ccg tgg tgg cgc gaa caa ggc ttc tcg ggc gtc ctc 2544
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
810 815 820

caa tcg agc tgt gac ccc atc tca ttt gcc aga gat acc agc atc gac 2592
Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
825 830 835 840

gtc gat cga caa tgg tcc att acc tgt ttc atg gtc gga gac ccg gga 2640
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
845 850 855

cgg aag tgg tcc caa cag tcc aag cag gta cga caa aag tct gtc tgg 2688
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
860 865 870

gac caa ctc cgc gca gcc tac gag aac gcc ggg gcc caa gtc cca gag 2736
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
875 880 885

ccg gcc aac gtg ctc gaa atc gag tgg tcg aag cag cag tat ttc caa 2784
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
890 895 900

gga gct ccg agc gcc gtc tat ggg ctg aac gat ctc atc aca ctg ggt 2832
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
905 910 915 920

tcg gcg ctc aga acg ccg ttc aag agt gtt cat ttc gtt gga acg gag 2880
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
925 930 935

acg tct tta gtt tgg aaa ggg tat atg gaa ggg gcc ata cga tcg ggt 2928
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
940 945 950

caa cga ggt gct gca gaa gtt gtg gct agc ctg gtg cca gca gca 2973
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Asp Leu Gly Gln Val Gln Gly Leu Ala Gly Asp Val Met Ser Phe Arg
10 15 20
Gly Ile Pro Tyr Ala Ala Pro Pro Val Gly Gly Leu Arg Trp Lys Pro
25 30 35 40
Pro Gln His Ala Arg Pro Trp Ala Gly Val Arg Pro Ala Thr Gln Phe
45 50 55
Gly Ser Asp Cys Phe Gly Ala Ala Tyr Leu Arg Lys Gly Ser Leu Ala
60 65 70
Pro Gly Val Ser Glu Asp Cys Leu Tyr Leu Asn Val Trp Ala Pro Ser
75 80 85
Gly Ala Lys Pro Gly Gln Tyr Pro Val Met Val Trp Val Tyr Gly Gly
90 95 100
Gly Phe Ala Gly Gly Thr Ala Ala Met Pro Tyr Tyr Asp Gly Glu Ala
105 110 115 120

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Leu Ala Arg Gln Gly Val Val Val Val Thr Phe Asn Tyr Arg Thr Asn
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Ile Leu Gly Phe Phe Ala His Pro Gly Leu Ser Arg Glu Ser Pro Thr
            140                 145                 150
Gly Thr Ser Gly Asn Tyr Gly Leu Leu Asp Ile Leu Ala Ala Leu Arg
        155                 160                 165
Trp Val Gln Ser Asn Ala Arg Ala Phe Gly Gly Asp Pro Gly Arg Val
    170                 175                 180
Thr Val Phe Gly Glu Ser Ala Gly Ala Ser Ala Ile Gly Leu Leu Leu
185                 190                 195                 200
Thr Ser Pro Leu Ser Lys Gly Leu Phe Arg Gly Ala Ile Leu Glu Ser
                205                 210                 215
Pro Gly Leu Thr Arg Pro Leu Ala Thr Leu Ala Asp Ser Ala Ala Ser
            220                 225                 230
Gly Glu Arg Leu Asp Ala Asp Leu Ser Arg Leu Arg Ser Thr Asp Pro
        235                 240                 245
Ala Thr Leu Met Ala Arg Ala Asp Ala Ala Arg Pro Ala Ser Arg Asp
    250                 255                 260
Leu Arg Arg Pro Arg Pro Thr Gly Pro Ile Val Asp Gly His Val Leu
265                 270                 275                 280
Pro Gln Thr Asp Ser Ala Ala Ile Ala Ala Gly Gln Leu Ala Pro Val
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Arg Val Leu Ile Gly Thr Asn Ala Asp Glu Gly Arg Ala Phe Leu Gly
            300                 305                 310
Arg Ala Pro Met Glu Thr Pro Ala Asp Tyr Gln Ala Tyr Leu Glu Ala
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Gln Phe Gly Asp Gln Ala Ala Ala Val Ala Ala Cys Tyr Pro Leu Asp
    330                 335                 340
Gly Arg Ala Thr Pro Lys Glu Met Val Ala Arg Ile Phe Gly Asp Asn
345                 350                 355                 360
Gln Phe Asn Arg Gly Val Ser Ala Phe Ser Glu Ala Leu Val Arg Gln
                365                 370                 375
Gly Ala Pro Val Trp Arg Tyr Gln Phe Asn Gly Asn Thr Glu Gly Gly
            380                 385                 390
Arg Ala Pro Ala Thr His Gly Ala Glu Ile Pro Tyr Val Phe Gly Val
        395                 400                 405
Phe Lys Leu Asp Glu Leu Gly Leu Phe Asp Trp Pro Pro Glu Gly Pro
    410                 415                 420
Thr Pro Ala Asp Arg Ala Leu Gly Gln Leu Met Ser Ser Ala Trp Val
425                 430                 435                 440
Arg Phe Ala Lys Asn Gly Asp Pro Ala Gly Asp Ala Leu Thr Trp Pro
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Ala Tyr Ser Thr Gly Lys Ser Thr Met Thr Phe Gly Pro Glu Gly Arg
            460                 465                 470
Ala Ala Val Val Ser Pro Gly Pro Ser Ile Pro Pro Cys Ala Asp Gly
        475                 480                 485
Ala Lys Ala Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser
    490                 495                 500
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
505                 510                 515                 520
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val
                525                 530                 535
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
            540                 545                 550
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
        555                 560                 565
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
    570                 575                 580
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
585                 590                 595                 600
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
                605                 610                 615
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Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
620 625 630
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
635 640 645
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
650 655 660
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
665 670 675 680
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
685 690 695
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
700 705 710
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala
715 720 725
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
730 735 740
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
745 750 755 760
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
765 770 775
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
780 785 790
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
795 800 805
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
810 815 820
Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
825 830 835 840
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
845 850 855
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
860 865 870
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
875 880 885
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
890 895 900
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
905 910 915 920
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
925 930 935
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act cga ctt ctt ttg gaa tat ctt gaa gaa aaa tat gaa gag cat ttg 96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
20 25 30

tat gag cgc gat gaa ggt gat aaa tgg cga aac aaa aag ttt gaa ttg 144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
35 40 45

ggt ttg gag ttt ccc aat ctt cct tat tat att gat ggt gat gtt aaa 192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
50 55 60

tta aca cag tct atg gcc atc ata cgt tat ata gct gac aag cac aac 240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65 70 75 80

atg ttg ggt ggt tgt cca aaa gag cgt gca gag att tca atg ctt gaa 288
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
85 90 95

gga gcg gtt ttg gat att aga tac ggt gtt tcg aga att gca tat agt 336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
100 105 110

aaa gac ttt gaa act ctc aaa gtt gat ttt ctt agc aag cta cct gaa 384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
115 120 125

atg ctg aaa atg ttc gaa gat cgt tta tgt cat aaa aca tat tta aat 432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
130 135 140

ggt gat cat gta acc cat cct gac ttc atg ttg tat gac gct ctt gat 480
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145 150 155 160

gtt gtt tta tac atg gac cca atg tgc ctg gat gcg ttc cca aaa tta 528
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
165 170 175

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gtt tgt ttt aaa aaa cgt att gaa gct atc cca caa att gat aag tac      576
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190

ttg aaa tcc agc aag tat ata gca tgg cct ttg cag ggc tgg caa gcc      624
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205

acg ttt ggt ggt ggc gac cat cct cca aaa tcg gat ctg gtt ccg cgt      672
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220

gga tcc ccg gaa ttc gct cct act gtc aag att gat gct ggg atg gtg      720
Gly Ser Pro Glu Phe Ala Pro Thr Val Lys Ile Asp Ala Gly Met Val
225                 230                 235                 240

gtc ggc acg act act act gtc ccc ggc acc act gcg acc gtc agc gag      768
Val Gly Thr Thr Thr Thr Val Pro Gly Thr Thr Ala Thr Val Ser Glu
                245                 250                 255

ttc ttg ggc gtt cct ttt gcc gcc tct ccg aca cga ttt gcg cct cct      816
Phe Leu Gly Val Pro Phe Ala Ala Ser Pro Thr Arg Phe Ala Pro Pro
            260                 265                 270

act cgt ccc gtg cct tgg tca acg cct ttg caa gcc act gca tat ggt      864
Thr Arg Pro Val Pro Trp Ser Thr Pro Leu Gln Ala Thr Ala Tyr Gly
        275                 280                 285

cca gca tgc cct caa caa ttc aat tac ccc gaa gaa ctc cgt gag att      912
Pro Ala Cys Pro Gln Gln Phe Asn Tyr Pro Glu Glu Leu Arg Glu Ile
    290                 295                 300

acg atg gcc tgg ttc aat aca ccg ccc ccg tca gct ggt gaa agt gag      960
Thr Met Ala Trp Phe Asn Thr Pro Pro Pro Ser Ala Gly Glu Ser Glu
305                 310                 315                 320

gac tgc ctg aac ctc aac atc tac gtc cca gga act gag aac aca aac     1008
Asp Cys Leu Asn Leu Asn Ile Tyr Val Pro Gly Thr Glu Asn Thr Asn
                325                 330                 335

aaa gcc gtc atg gtt tgg ata tac ggt gga gcg ctg gaa tat ggt tgg     1056
Lys Ala Val Met Val Trp Ile Tyr Gly Gly Ala Leu Glu Tyr Gly Trp
            340                 345                 350

aat tca ttc cac ctt tac gac ggg gct agt ttc gca gcc aat cag gat     1104
Asn Ser Phe His Leu Tyr Asp Gly Ala Ser Phe Ala Ala Asn Gln Asp
        355                 360                 365

gtc atc gcc gtg acc atc aac tac aga acg aac att ctg ggg ttc cct     1152
Val Ile Ala Val Thr Ile Asn Tyr Arg Thr Asn Ile Leu Gly Phe Pro
    370                 375                 380

gct gcc cct cag ctt cca ata aca cag cga aat ctg ggg ttc cta gac     1200
Ala Ala Pro Gln Leu Pro Ile Thr Gln Arg Asn Leu Gly Phe Leu Asp
385                 390                 395                 400

caa agg ttt gct ttg gat tgg gta cag cgg aac atc gca gcc ttt ggc     1248
Gln Arg Phe Ala Leu Asp Trp Val Gln Arg Asn Ile Ala Ala Phe Gly
                405                 410                 415

ggt gat cct cga aag gtc aca ata ttt ggg cag agt gcg ggg ggc aga     1296
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Gly Asp Pro Arg Lys Val Thr Ile Phe Gly Gln Ser Ala Gly Gly Arg
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agt gtc gac gtc ctc ttg acg tct atg cca cac aac cca ccc ttc cga       1344
Ser Val Asp Val Leu Leu Thr Ser Met Pro His Asn Pro Pro Phe Arg
        435                 440                 445

gca gca atc atg gag tcc ggt gtg gct aac tac aac ttc ccc aag gga       1392
Ala Ala Ile Met Glu Ser Gly Val Ala Asn Tyr Asn Phe Pro Lys Gly
    450                 455                 460

gat ttg tcc gaa cct tgg aac acc act gtt caa gct ctc aac tgt acc       1440
Asp Leu Ser Glu Pro Trp Asn Thr Thr Val Gln Ala Leu Asn Cys Thr
465                 470                 475                 480

acc agt atc gac atc ttg agt tgt atg aga aga gtc gat ctc gcc act       1488
Thr Ser Ile Asp Ile Leu Ser Cys Met Arg Arg Val Asp Leu Ala Thr
                485                 490                 495

ctg atg aac acg atc gag caa ctc gga ctt ggg ttt gag tac acg ttg       1536
Leu Met Asn Thr Ile Glu Gln Leu Gly Leu Gly Phe Glu Tyr Thr Leu
            500                 505                 510

gac aac gta acg gct gtg tac cgt tct gaa acg gct cgc acg act ggt       1584
Asp Asn Val Thr Ala Val Tyr Arg Ser Glu Thr Ala Arg Thr Thr Gly
        515                 520                 525

gac att gct cgt gta cct gtt ctc gtc ggg acg gtg gcc aac gac gga       1632
Asp Ile Ala Arg Val Pro Val Leu Val Gly Thr Val Ala Asn Asp Gly
    530                 535                 540

ctt ctc ttt gtc ctc ggg gag aat gac acc caa gca tat ctc gag gag       1680
Leu Leu Phe Val Leu Gly Glu Asn Asp Thr Gln Ala Tyr Leu Glu Glu
545                 550                 555                 560

gca atc ccg aat cag ccc gac ctt tac cag act ctc ctt gga gca tat       1728
Ala Ile Pro Asn Gln Pro Asp Leu Tyr Gln Thr Leu Leu Gly Ala Tyr
                565                 570                 575

ccc att gga tcc cca ggg atc gga tcg cct caa gat cag att gcc gcc       1776
Pro Ile Gly Ser Pro Gly Ile Gly Ser Pro Gln Asp Gln Ile Ala Ala
            580                 585                 590

att gag acc gag gta aga ttc cag tgt cct tct gcc atc gtg gct cag       1824
Ile Glu Thr Glu Val Arg Phe Gln Cys Pro Ser Ala Ile Val Ala Gln
        595                 600                 605

gac tcc cgg aat cgg ggt atc cct tct tgg cgc tac tac tac aat gcg       1872
Asp Ser Arg Asn Arg Gly Ile Pro Ser Trp Arg Tyr Tyr Tyr Asn Ala
    610                 615                 620

acc ttt gag aat ctg gag ctt ttc cct ggg tcc gaa gtg tac cac agc       1920
Thr Phe Glu Asn Leu Glu Leu Phe Pro Gly Ser Glu Val Tyr His Ser
625                 630                 635                 640

tct gaa gtc ggg atg gtg ttt ggc acg tat cct gtc gca agt gcg acc       1968
Ser Glu Val Gly Met Val Phe Gly Thr Tyr Pro Val Ala Ser Ala Thr
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gcc ttg gag gcc cag acg agc aaa tac atg cag ggt gcc tgg gcg gcc       2016
Ala Leu Glu Ala Gln Thr Ser Lys Tyr Met Gln Gly Ala Trp Ala Ala
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        675                 680                 685

gtc gcg gcg ctt ggc tca cca ggc aaa gcc atc cag gtt gac gtc tct      2112
Val Ala Ala Leu Gly Ser Pro Gly Lys Ala Ile Gln Val Asp Val Ser
    690                 695                 700

cca gcg aca ata gac caa cga tgt gcc ttg tac acg cgt tat tat act      2160
Pro Ala Thr Ile Asp Gln Arg Cys Ala Leu Tyr Thr Arg Tyr Tyr Thr
705                 710                 715                     720

gag ttg ggc aca atc gcg ccg agg aca ttt ggc gga ggc agc ggc gga      2208
Glu Leu Gly Thr Ile Ala Pro Arg Thr Phe Gly Gly Gly Ser Gly Gly
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Gly Ser Gly Gly Gly Ser Lys Asp Asn Val Ala Asp Val Val Val Val
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Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln Ala Ala
        755                 760                 765

ggt ctg tcc tgc ctc gtt ctt gag gcg atg gat cgt gta ggg gga aag      2352
Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly Gly Lys
    770                 775                 780

act ctg agc gta caa tcg ggt ccc ggc agg acg act atc aac gac ctc      2400
Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu
785                 790                 795                     800

ggc gct gcg tgg atc aat gac agc aac caa agc gaa gta tcc aga ttg      2448
Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Ser Arg Leu
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ttt gaa aga ttt cat ttg gag ggc gag ctc cag agg acg act gga aat      2496
Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn
            820                 825                 830

tca atc cat caa gca caa gac ggt aca acc act aca gct cct tat ggt      2544
Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly
        835                 840                 845

gac tcc ttg ctg agc gag gag gtt gca agt gca ctt gcg gaa ctc ctc      2592
Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu Leu Leu
    850                 855                 860

ccc gta tgg tct cag ctg atc gaa gag cat agc ctt caa gac ctc aag      2640
Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln Asp Leu Lys
865                 870                 875                     880

gcg agc cct cag gcg aag cgg ctc gac agt gtg agc ttc gcg cac tac      2688
Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe Ala His Tyr
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tgt gag aag gaa cta aac ttg cct gct gtt ctc ggc gta gca aac cag      2736
Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val Ala Asn Gln
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atc aca cgc gct ctg ctc ggt gtg gaa gcc cac gag atc agc atg ctt      2784
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Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe
930 935 940

tcg gac aag aaa gac ggc ggg cag tat atg cga tgc aaa aca ggt atg 2880
Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr Gly Met
945 950 955 960

cag tcg att tgc cat gcc atg tca aag gaa ctt gtt cca ggc tca gtg 2928
Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro Gly Ser Val
965 970 975

cac ctc aac acc ccc gtc gct gaa att gag cag tcg gca tcc ggc tgt 2976
His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys
980 985 990

aca gta cga tcg gcc tcg ggc gcc gtg ttc cga agc aaa aag gtg gtg 3024
Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys Lys Val Val
995 1000 1005

gtt tcg tta ccg aca acc ttg tat ccc acc ttg aca ttt tca cca cct 3072
Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro
1010 1015 1020

ctt ccc gcc gag aag caa gca ttg gcg gaa aat tct atc ctg ggc tac 3120
Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr
1025 1030 1035 1040

tat agc aag ata gtc ttc gta tgg gac aag ccg tgg tgg cgc gaa caa 3168
Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg Glu Gln
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ggc ttc tcg ggc gtc ctc caa tcg agc tgt gac ccc atc tca ttt gcc 3216
Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala
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aga gat acc agc atc gac gtc gat cga caa tgg tcc att acc tgt ttc 3264
Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile Thr Cys Phe
1075 1080 1085

atg gtc gga gac ccg gga cgg aag tgg tcc caa cag tcc aag cag gta 3312
Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys Gln Val
1090 1095 1100

cga caa aag tct gtc tgg gac caa ctc cgc gca gcc tac gag aac gcc 3360
Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala
1105 1110 1115 1120

ggg gcc caa gtc cca gag ccg gcc aac gtg ctc gaa atc gag tgg tcg 3408
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1125 1130 1135

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Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn
1140 1145 1150

gat ctc atc aca ctg ggt tcg gcg ctc aga acg ccg ttc aag agt gtt 3504
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1155 1160 1165

cat ttc gtt gga acg gag acg tct tta gtt tgg aaa ggg tat atg gaa 3552
His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr Met Glu
1170 1175 1180

ggg gcc ata cga tcg ggt caa cga ggt gct gca gaa gtt gtg gct agc 3600
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35 40 45
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
50 55 60
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
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Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
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Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
100 105 110
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
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Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
130 135 140
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145 150 155 160
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
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Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
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Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
195 200 205
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
210 215 220
Gly Ser Pro Glu Phe Ala Pro Thr Val Lys Ile Asp Ala Gly Met Val
225 230 235 240
Val Gly Thr Thr Thr Thr Val Pro Gly Thr Thr Ala Thr Val Ser Glu
245 250 255
Phe Leu Gly Val Pro Phe Ala Ala Ser Pro Thr Arg Phe Ala Pro Pro
260 265 270
Thr Arg Pro Val Pro Trp Ser Thr Pro Leu Gln Ala Thr Ala Tyr Gly
275 280 285
Pro Ala Cys Pro Gln Gln Phe Asn Tyr Pro Glu Glu Leu Arg Glu Ile
290 295 300
Thr Met Ala Trp Phe Asn Thr Pro Pro Pro Ser Ala Gly Glu Ser Glu
305 310 315 320
Asp Cys Leu Asn Leu Asn Ile Tyr Val Pro Gly Thr Glu Asn Thr Asn
325 330 335

Lys Ala Val Met Val Trp Ile Tyr Gly Gly Ala Leu Glu Tyr Gly Trp
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Asn Ser Phe His Leu Tyr Asp Gly Ala Ser Phe Ala Ala Asn Gln Asp
        355   360   365
Val Ile Ala Val Thr Ile Asn Tyr Arg Thr Asn Ile Leu Gly Phe Pro
    370   375   380
Ala Ala Pro Gln Leu Pro Ile Thr Gln Arg Asn Leu Gly Phe Leu Asp
385   390   395   400
Gln Arg Phe Ala Leu Asp Trp Val Gln Arg Asn Ile Ala Ala Phe Gly
                405   410   415
Gly Asp Pro Arg Lys Val Thr Ile Phe Gly Gln Ser Ala Gly Gly Arg
            420   425   430
Ser Val Asp Val Leu Leu Thr Ser Met Pro His Asn Pro Pro Phe Arg
        435   440   445
Ala Ala Ile Met Glu Ser Gly Val Ala Asn Tyr Asn Phe Pro Lys Gly
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Asp Leu Ser Glu Pro Trp Asn Thr Thr Val Gln Ala Leu Asn Cys Thr
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Thr Ser Ile Asp Ile Leu Ser Cys Met Arg Arg Val Asp Leu Ala Thr
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Leu Met Asn Thr Ile Glu Gln Leu Gly Leu Gly Phe Glu Tyr Thr Leu
            500   505   510
Asp Asn Val Thr Ala Val Tyr Arg Ser Glu Thr Ala Arg Thr Thr Gly
        515   520   525
Asp Ile Ala Arg Val Pro Val Leu Val Gly Thr Val Ala Asn Asp Gly
    530   535   540
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545   550   555   560
Ala Ile Pro Asn Gln Pro Asp Leu Tyr Gln Thr Leu Leu Gly Ala Tyr
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Pro Ile Gly Ser Pro Gly Ile Gly Ser Pro Gln Asp Gln Ile Ala Ala
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Asp Ser Arg Asn Arg Gly Ile Pro Ser Trp Arg Tyr Tyr Tyr Asn Ala
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Thr Phe Glu Asn Leu Glu Leu Phe Pro Gly Ser Glu Val Tyr His Ser
625   630   635   640
Ser Glu Val Gly Met Val Phe Gly Thr Tyr Pro Val Ala Ser Ala Thr
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Ala Leu Glu Ala Gln Thr Ser Lys Tyr Met Gln Gly Ala Trp Ala Ala
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Phe Ala Lys Asn Pro Met Asn Gly Pro Gly Trp Lys Gln Val Pro Asn
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Val Ala Ala Leu Gly Ser Pro Gly Lys Ala Ile Gln Val Asp Val Ser
    690   695   700
Pro Ala Thr Ile Asp Gln Arg Cys Ala Leu Tyr Thr Arg Tyr Tyr Thr
705   710   715   720
Glu Leu Gly Thr Ile Ala Pro Arg Thr Phe Gly Gly Gly Ser Gly Gly
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Gly Ser Gly Gly Gly Ser Lys Asp Asn Val Ala Asp Val Val Val Val
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Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln Ala Ala
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Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly Gly Lys
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Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu
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Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Ser Arg Leu
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Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn
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Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly
        835   840   845
Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu Leu Leu
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Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln Asp Leu Lys
865   870   875   880
Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe Ala His Tyr
                885   890   895
Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val Ala Asn Gln
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His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys
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Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg Glu Gln
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Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys Gln Val
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Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala
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Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile Glu Trp Ser
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Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn
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Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe Lys Ser Val
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His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr Met Glu
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act cga ctt ctt ttg gaa tat ctt gaa gaa aaa tat gaa gag cat ttg      96
Thr Arg Leu Leu Leu Glu Tyr Leu Glu Glu Lys Tyr Glu Glu His Leu
            20                  25                  30

tat gag cgc gat gaa ggt gat aaa tgg cga aac aaa aag ttt gaa ttg     144
Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45

ggt ttg gag ttt ccc aat ctt cct tat tat att gat ggt gat gtt aaa     192
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60

tta aca cag tct atg gcc atc ata cgt tat ata gct gac aag cac aac     240
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80

atg ttg ggt ggt tgt cca aaa gag cgt gca gag att tca atg ctt gaa     288
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                85                  90                  95

gga gcg gtt ttg gat att aga tac ggt gtt tcg aga att gca tat agt     336
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110

aaa gac ttt gaa act ctc aaa gtt gat ttt ctt agc aag cta cct gaa     384
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125

atg ctg aaa atg ttc gaa gat cgt tta tgt cat aaa aca tat tta aat     432
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140

ggt gat cat gta acc cat cct gac ttc atg ttg tat gac gct ctt gat     480
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Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
165 170 175

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Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
180 185 190

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225 230 235 240

cag gtt cag gga ctg gcc ggg gac gtg atg agc ttt cgc gga ata ccc 768
Gln Val Gln Gly Leu Ala Gly Asp Val Met Ser Phe Arg Gly Ile Pro
245 250 255

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Tyr Ala Ala Pro Pro Val Gly Gly Leu Arg Trp Lys Pro Pro Gln His
260 265 270

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Ala Arg Pro Trp Ala Gly Val Arg Pro Ala Thr Gln Phe Gly Ser Asp
275 280 285

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340 345 350

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355 360 365

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530 535 540

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Met Glu Thr Pro Ala Asp Tyr Gln Ala Tyr Leu Glu Ala Gln Phe Gly
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Asp Gln Ala Ala Ala Val Ala Ala Cys Tyr Pro Leu Asp Gly Arg Ala
565 570 575

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580 585 590

cgg ggg gtc tcg gcc ttc tcg gaa gcg ctt gtg cgc cag ggc gcg ccc 1824
Arg Gly Val Ser Ala Phe Ser Glu Ala Leu Val Arg Gln Gly Ala Pro
595 600 605

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610 615 620

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Ala Thr His Gly Ala Glu Ile Pro Tyr Val Phe Gly Val Phe Lys Leu
625 630 635 640

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645 650 655

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660 665 670

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Lys Asn Gly Asp Pro Ala Gly Asp Ala Leu Thr Trp Pro Ala Tyr Ser
675 680 685

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725 730 735

gtt gcg gac gtg gta gtg gtg ggc gct ggc ttg agc ggt ttg gag acg 2256
Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr
740 745 750

gca cgc aaa gtc cag gcc gcc ggt ctg tcc tgc ctc gtt ctt gag gcg 2304
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755 760 765

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770 775 780

agg acg act atc aac gac ctc ggc gct gcg tgg atc aat gac agc aac 2400
Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn
785 790 795 800

caa agc gaa gta tcc aga ttg ttt gaa aga ttt cat ttg gag ggc gag 2448
Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu
805 810 815

ctc cag agg acg act gga aat tca atc cat caa gca caa gac ggt aca 2496
Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr
820 825 830

acc act aca gct cct tat ggt gac tcc ttg ctg agc gag gag gtt gca 2544
Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala
835 840 845

agt gca ctt gcg gaa ctc ctc ccc gta tgg tct cag ctg atc gaa gag 2592
Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu
850 855 860

cat agc ctt caa gac ctc aag gcg agc cct cag gcg aag cgg ctc gac 2640
His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp
865 870 875 880

agt gtg agc ttc gcg cac tac tgt gag aag gaa cta aac ttg cct gct 2688
Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala
885 890 895

gtt ctc ggc gta gca aac cag atc aca cgc gct ctg ctc ggt gtg gaa 2736
Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu
900 905 910

gcc cac gag atc agc atg ctt ttt ctc acc gac tac atc aag agt gcc 2784
Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala
915 920 925

acc ggt ctc agt aat att ttc tcg gac aag aaa gac ggc ggg cag tat 2832
Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr
930 935 940

atg cga tgc aaa aca ggt atg cag tcg att tgc cat gcc atg tca aag 2880
Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys
945 950 955 960

gaa ctt gtt cca ggc tca gtg cac ctc aac acc ccc gtc gct gaa att 2928
Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile
965 970 975

gag cag tcg gca tcc ggc tgt aca gta cga tcg gcc tcg ggc gcc gtg 2976
Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val
980 985 990

ttc cga agc aaa aag gtg gtg gtt tcg tta ccg aca acc ttg tat ccc 3024
Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro
995 1000 1005

acc ttg aca ttt tca cca cct ctt ccc gcc gag aag caa gca ttg gcg 3072
Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala
1010 1015 1020

gaa aat tct atc ctg ggc tac tat agc aag ata gtc ttc gta tgg gac 3120
Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp
1025 1030 1035 1040

aag ccg tgg tgg cgc gaa caa ggc ttc tcg ggc gtc ctc caa tcg agc 3168
Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser
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tgt gac ccc atc tca ttt gcc aga gat acc agc atc gac gtc gat cga 3216
Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg
1060 1065 1070

caa tgg tcc att acc tgt ttc atg gtc gga gac ccg gga cgg aag tgg 3264
Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp
1075 1080 1085

tcc caa cag tcc aag cag gta cga caa aag tct gtc tgg gac caa ctc 3312
Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu
1090 1095 1100

cgc gca gcc tac gag aac gcc ggg gcc caa gtc cca gag ccg gcc aac 3360
Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn
1105 1110 1115 1120

gtg ctc gaa atc gag tgg tcg aag cag cag tat ttc caa gga gct ccg 3408
Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro
1125 1130 1135

agc gcc gtc tat ggg ctg aac gat ctc atc aca ctg ggt tcg gcg ctc 3456

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Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu
            1140                1145                1150

aga acg ccg ttc aag agt gtt cat ttc gtt gga acg gag acg tct tta      3504
Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu
        1155                1160                1165

gtt tgg aaa ggg tat atg gaa ggg gcc ata cga tcg ggt caa cga ggt      3552
Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly
    1170                1175                1180

gct gca gaa gtt gtg gct agc ctg gtg cca gca gca tag                  3591
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1185                1190                1195


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Tyr Glu Arg Asp Glu Gly Asp Lys Trp Arg Asn Lys Lys Phe Glu Leu
        35                  40                  45
Gly Leu Glu Phe Pro Asn Leu Pro Tyr Tyr Ile Asp Gly Asp Val Lys
    50                  55                  60
Leu Thr Gln Ser Met Ala Ile Ile Arg Tyr Ile Ala Asp Lys His Asn
65                  70                  75                  80
Met Leu Gly Gly Cys Pro Lys Glu Arg Ala Glu Ile Ser Met Leu Glu
                85                  90                  95
Gly Ala Val Leu Asp Ile Arg Tyr Gly Val Ser Arg Ile Ala Tyr Ser
            100                 105                 110
Lys Asp Phe Glu Thr Leu Lys Val Asp Phe Leu Ser Lys Leu Pro Glu
        115                 120                 125
Met Leu Lys Met Phe Glu Asp Arg Leu Cys His Lys Thr Tyr Leu Asn
    130                 135                 140
Gly Asp His Val Thr His Pro Asp Phe Met Leu Tyr Asp Ala Leu Asp
145                 150                 155                 160
Val Val Leu Tyr Met Asp Pro Met Cys Leu Asp Ala Phe Pro Lys Leu
                165                 170                 175
Val Cys Phe Lys Lys Arg Ile Glu Ala Ile Pro Gln Ile Asp Lys Tyr
            180                 185                 190
Leu Lys Ser Ser Lys Tyr Ile Ala Trp Pro Leu Gln Gly Trp Gln Ala
        195                 200                 205
Thr Phe Gly Gly Gly Asp His Pro Pro Lys Ser Asp Leu Val Pro Arg
    210                 215                 220
Gly Ser Pro Glu Phe Thr Asp Phe Pro Val Arg Arg Thr Asp Leu Gly
225                 230                 235                 240
Gln Val Gln Gly Leu Ala Gly Asp Val Met Ser Phe Arg Gly Ile Pro
                245                 250                 255
Tyr Ala Ala Pro Pro Val Gly Gly Leu Arg Trp Lys Pro Pro Gln His
            260                 265                 270
Ala Arg Pro Trp Ala Gly Val Arg Pro Ala Thr Gln Phe Gly Ser Asp
        275                 280                 285
Cys Phe Gly Ala Ala Tyr Leu Arg Lys Gly Ser Leu Ala Pro Gly Val
    290                 295                 300
Ser Glu Asp Cys Leu Tyr Leu Asn Val Trp Ala Pro Ser Gly Ala Lys
305                 310                 315                 320
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Pro Gly Gln Tyr Pro Val Met Val Trp Val Tyr Gly Gly Gly Phe Ala
325 330 335

Gly Gly Thr Ala Ala Met Pro Tyr Tyr Asp Gly Glu Ala Leu Ala Arg
340 345 350

Gln Gly Val Val Val Val Thr Phe Asn Tyr Arg Thr Asn Ile Leu Gly
355 360 365

Phe Phe Ala His Pro Gly Leu Ser Arg Glu Ser Pro Thr Gly Thr Ser
370 375 380

Gly Asn Tyr Gly Leu Leu Asp Ile Leu Ala Ala Leu Arg Trp Val Gln
385 390 395 400

Ser Asn Ala Arg Ala Phe Gly Gly Asp Pro Gly Arg Val Thr Val Phe
405 410 415

Gly Glu Ser Ala Gly Ala Ser Ala Ile Gly Leu Leu Leu Thr Ser Pro
420 425 430

Leu Ser Lys Gly Leu Phe Arg Gly Ala Ile Leu Glu Ser Pro Gly Leu
435 440 445

Thr Arg Pro Leu Ala Thr Leu Ala Asp Ser Ala Ala Ser Gly Glu Arg
450 455 460

Leu Asp Ala Asp Leu Ser Arg Leu Arg Ser Thr Asp Pro Ala Thr Leu
465 470 475 480

Met Ala Arg Ala Asp Ala Ala Arg Pro Ala Ser Arg Asp Leu Arg Arg
485 490 495

Pro Arg Pro Thr Gly Pro Ile Val Asp Gly His Val Leu Pro Gln Thr
500 505 510

Asp Ser Ala Ala Ile Ala Ala Gly Gln Leu Ala Pro Val Arg Val Leu
515 520 525

Ile Gly Thr Asn Ala Asp Glu Gly Arg Ala Phe Leu Gly Arg Ala Pro
530 535 540

Met Glu Thr Pro Ala Asp Tyr Gln Ala Tyr Leu Glu Ala Gln Phe Gly
545 550 555 560

Asp Gln Ala Ala Ala Val Ala Ala Cys Tyr Pro Leu Asp Gly Arg Ala
565 570 575

Thr Pro Lys Glu Met Val Ala Arg Ile Phe Gly Asp Asn Gln Phe Asn
580 585 590

Arg Gly Val Ser Ala Phe Ser Glu Ala Leu Val Arg Gln Gly Ala Pro
595 600 605

Val Trp Arg Tyr Gln Phe Asn Gly Asn Thr Glu Gly Gly Arg Ala Pro
610 615 620

Ala Thr His Gly Ala Glu Ile Pro Tyr Val Phe Gly Val Phe Lys Leu
625 630 635 640

Asp Glu Leu Gly Leu Phe Asp Trp Pro Pro Glu Gly Pro Thr Pro Ala
645 650 655

Asp Arg Ala Leu Gly Gln Leu Met Ser Ser Ala Trp Val Arg Phe Ala
660 665 670

Lys Asn Gly Asp Pro Ala Gly Asp Ala Leu Thr Trp Pro Ala Tyr Ser
675 680 685

Thr Gly Lys Ser Thr Met Thr Phe Gly Pro Glu Gly Arg Ala Ala Val
690 695 700

Val Ser Pro Gly Pro Ser Ile Pro Pro Cys Ala Asp Gly Ala Lys Ala
705 710 715 720

Gly Gly Gly Gly Ser Gly Gly Gly Ser Gly Gly Gly Ser Lys Asp Asn
725 730 735

Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr
740 745 750

Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala
755 760 765

Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly
770 775 780

Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn
785 790 795 800

Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu
805 810 815

Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr
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Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala
        835   840   845
Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu
    850   855   860
His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp
865   870   875   880
Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala
                885   890   895
Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu
            900   905   910
Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala
        915   920   925
Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr
    930   935   940
Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys
945   950   955   960
Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile
                965   970   975
Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val
            980   985   990
Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro
        995   1000   1005
Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala
    1010   1015   1020
Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp
1025   1030   1035   1040
Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser
                1045   1050   1055
Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg
            1060   1065   1070
Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp
        1075   1080   1085
Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu
    1090   1095   1100
Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn
1105   1110   1115   1120
Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro
                1125   1130   1135
Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu
            1140   1145   1150
Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu
        1155   1160   1165
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Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala
1185   1190   1195

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glycosylation sites

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Ala Gly Tyr Ser His Val Gly Val Gly Pro Asp Gly Gly Arg Tyr Val
             20                  25                  30

aca ata gct gga cag att gga caa gac gct tcg ggc gtg aca gac cct      144
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Gly Val Thr Asp Pro
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gcc tac gag aaa cag gtt gcc caa gca ttc gcc aat ctg cga gct tgc      192
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
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ctt gct gca gtt gga gcc act tca aac gac gtc acc aag ctc aat tac      240
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Val Thr Lys Leu Asn Tyr
 65                  70                  75                  80

tac atc gtc gac tac gcc ccg agc aaa ctc acc gca att gga gat ggg      288
Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
                 85                  90                  95

ctg aag gct acc ttt gcc ctt gac agg ctc cct cct tgc acg ctg gtg      336
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
            100                 105                 110

cca gtg tcg gcc ttg tct tca cct gaa tac ctc ttt gag gtt gat gcc      384
Pro Val Ser Ala Leu Ser Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
        115                 120                 125

acg gcg ctg gtg ccg gga cac acg acc cca gac aac gtt gcg gac gtg      432
Thr Ala Leu Val Pro Gly His Thr Thr Pro Asp Asn Val Ala Asp Val
    130                 135                 140

gta gtg gtg ggc gct ggc ttg agc ggt ttg gag acg gca cgc aaa gtc      480
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145                 150                 155                 160

cag gcc gcc ggt ctg tcc tgc ctc gtt ctt gag gcg atg gat cgt gta      528
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
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ggg gga aag act ctg agc gta caa tcg ggt ccc ggc agg acg act atc      576
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
            180                 185                 190

aac gac ctc ggc gct gcg tgg atc aat gat agc aat cag gcc gaa gta      624
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ala Glu Val
        195                 200                 205

tcc aga ttg ttt gaa aga ttt cat ttg gag ggc gag ctc cag agg acg      672
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
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act gga aat tca atc cat caa gca caa gac ggt aca acc act aca gct      720
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
225                 230                 235                 240

cct tat ggt gac tcc ttg ctg agc gag gag gtt gca agt gca ctt gcg      768
Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
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245 250 255

gaa ctc ctc ccc gta tgg tct cag ctg atc gaa gag cat agc ctt caa 816
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260 265 270

gac ctc aag gcg agc cct cag gcg aag cgg ctc gac agt gtg agc ttc 864
Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
275 280 285

gcg cac tac tgt gag aag gaa cta aac ttg cct gct gtt ctc ggc gta 912
Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val
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gca aac cag atc aca cgc gct ctg ctc ggt gtg gaa gcc cac gag atc 960
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
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Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
325 330 335

aat att ttc tcg gac aag aaa gac ggc ggg cag tat atg cga tgc aaa 1056
Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
340 345 350

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Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
355 360 365

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Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala
370 375 380

tcc ggc tgt aca gta cga tcg gcc tcg ggc gcc gtg ttc cga agc aaa 1200
Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
385 390 395 400

aag gtg gtg gtt tcg tta ccg aca acc ttg tat ccc acc ttg aca ttt 1248
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
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Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile
420 425 430

ctg ggc tac tat agc aag ata gtc ttc gta tgg gac aag ccg tgg tgg 1344
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp
435 440 445

cgc gaa caa ggc ttc tcg ggc gtc ctc caa tcg agc tgt gac ccc atc 1392
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
450 455 460

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Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile
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Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
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aag cag gta cga caa aag tct gtc tgg gac caa ctc cgc gca gcc tac 1536
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
500 505 510

gag aac gcc ggg gcc caa gtc cca gag ccg gcc aac gtg ctc gaa atc 1584
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
515 520 525

gag tgg tcg aag cag cag tat ttc caa gga gct ccg agc gcc gtc tat 1632
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
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545 550 555 560

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Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
565 570 575

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Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val
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35 40 45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
50 55 60
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Val Thr Lys Leu Asn Tyr
65 70 75 80
Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
85 90 95
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
100 105 110
Pro Val Ser Ala Leu Ser Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
115 120 125
Thr Ala Leu Val Pro Gly His Thr Thr Pro Asp Asn Val Ala Asp Val
130 135 140
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145 150 155 160
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
165 170 175
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
180 185 190
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ala Glu Val
195 200 205
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr

210 215 220
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
225 230 235 240
Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
245 250 255
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln
260 265 270
Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
275 280 285
Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val
290 295 300
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
305 310 315 320
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
325 330 335
Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
340 345 350
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
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Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala
370 375 380
Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
385 390 395 400
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
405 410 415
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Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp
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Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
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Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile
465 470 475 480
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
485 490 495
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
500 505 510
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
515 520 525
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
530 535 540
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
545 550 555 560
Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
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agaatacagt cactgactcc acttcgtcca gctgagcgag gaggttgcaa gtgcacttgc     840
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taatattgtc tcggacaaga aagacggcgg gcagtatatg cgatgcaaaa caggtgcgtg    1140
cggtgtcctc tcaggtaggg gactcgtttc ttagtggtca ttccaggtat gcagtcgatt    1200
tgccatgcca tgtcaaagga acttgttcca ggctcagtgc acctcaacac ccccgtcgct    1260
ggaattgagc agtcggcgtc cggctgtata gtacgatcgg cctcgggcgc cgtgttccga    1320
agcaaaaagg tggtggtttc gttaccgaca acattgtatc ccaccttgac attttcacca    1380
cctcttcccg ccgagaagca agcattggcg gaaaaatcta tcctcggcta ctatagcaag    1440
atagtcttcg tatgggacaa cccgtggtgg cgcgaacaag gcttctcggg cgtcctccaa    1500
tcgagctgtg accccatctc atttgccaga gataccagca tcgaagtcga tcggcaatgg    1560
tccattacct gtttcatggt cggagacccg ggacggaagt ggtcccaaca gtccaagcag    1620
gtacgacaaa agtctgtctg ggaccaactc cgcgcagcct acgagaacgc cggggcccaa    1680
gtcccagagc cggccaacgt gctcgaaatc gagtggtcga agcagcagta tttccaagga    1740
gctccgagcg ccgtctatgg gctgaacgat ctcatcacac tgggttcggc gctcagaacg    1800
ccgttcaagt gtgttcattt cgttggaacg gagacgtctt tagtttggaa agggtatatg    1860
gaaggggcca tacgatcggg tcaacgaggt gctgcagaag ttgtggctag cctggtgcca    1920
gcagcatag                                                            1929
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<210> 36
<211> 600
<212> PRT
<213> Exophiala spinifera

<400> 36
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 1               5                  10                  15
Ala Gly Tyr Ser His Ile Gly Val Gly Pro Asn Glu Ala Arg Tyr Val
            20                  25                  30
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Leu Gly Val Thr Asp Pro
        35                  40                  45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
    50                  55                  60
Leu Ala Ala Val Gly Ala Ser Ser Asn Asp Val Thr Lys Leu Asn Tyr
65                  70                  75                  80
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Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
                85                  90                  95
Leu Lys Ser Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
            100                 105                 110
Pro Val Pro Ala Leu Ala Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
        115                 120                 125
Thr Ala Leu Val Pro Gly His Ser Thr Pro Asp Asn Val Ala Asp Val
    130                 135                 140
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145                 150                 155                 160
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
                165                 170                 175
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
            180                 185                 190
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
        195                 200                 205
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
    210                 215                 220
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
225                 230                 235                 240
Pro Tyr Gly Asp Ser Pro Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
                245                 250                 255
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu Tyr Ser Leu Glu
            260                 265                 270
Asp Pro Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
        275                 280                 285
Ala His Tyr Cys Glu Lys Asp Leu Asn Leu Pro Ala Val Leu Ser Val
    290                 295                 300
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
305                 310                 315                 320
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
                325                 330                 335
Asn Ile Val Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
            340                 345                 350
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
        355                 360                 365
Gly Ser Val His Leu Asn Thr Pro Val Ala Gly Ile Glu Gln Ser Ala
    370                 375                 380
Ser Gly Cys Ile Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
385                 390                 395                 400
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
                405                 410                 415
Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Lys Ser Ile
            420                 425                 430
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Asn Pro Trp Trp
        435                 440                 445
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
    450                 455                 460
Ser Phe Ala Arg Asp Thr Ser Ile Glu Val Asp Arg Gln Trp Ser Ile
465                 470                 475                 480
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
                485                 490                 495
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
            500                 505                 510
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
        515                 520                 525
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
    530                 535                 540
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
545                 550                 555                 560
Lys Cys Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
                565                 570                 575
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Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val
580 585 590
Val Ala Ser Leu Val Pro Ala Ala
595 600

<210> 37
<211> 1929
<212> DNA
<213> Exophiala spinifera

<220>
<221> intron
<222> (739)...(811)

<221> intron
<222> (1134)...(1186)

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cacatcggcg taggcccaaa cgaagcgagg tatgtgacaa tagctggaca gattggacaa 120
gacgctttgg gcgtgacaga cccagcctac gagaaacagg ttgcccaagc attcgccaat 180
ctgcgagctt gccttgctgc agttggagcc tcttcaaacg acgtcaccaa gctcaattac 240
tacatcgtcg actacgcccc gagcaaactc accgcaattg gagatgggct gaagtctacc 300
tttgcccttg acaggctccc tccttgcacg ctggtgccag taccggcctt ggcttcacct 360
gaatacctct ttgaggttga cgccacggcg ctggtgccag gacactcgac cccagacaac 420
gttgcggacg tggtagtggt gggcgctggc ttgagcggct tggagacggc acgcaaagtc 480
caggccgccg gtctgtcctg cctcgttctt gaggcgatgg atcgtgtagg gggaaagact 540
ctgagcgtac aatcgggtcc cggcaggacg actatcaacg acctcggcgc tgcgtggatc 600
aatgacagca accaaagcga agtatccaga ttgtttgaaa gatttcattt ggagggcgag 660
ctccagagga cgaccggaaa ttcaatccat caagcacaag acggtacaac cactacagct 720
ccttatggtg actccccggt aagcacaatc ccactttgtg atgagacctc tgtcgagtgt 780
agaatacagt cactgactcc acttcgtcca gctgagcgag gaggttgcaa gtgcacttgc 840
ggaactcctc cccgtatggt ctcagctgat cgaagagtat agccttgaag accccaaggc 900
gagccctcag gcgaagcggc tcgacagtgt gagcttcgcg cactactgtg agaaggacct 960
aaacttgcct gctgttctca gcgtggcaaa ccagatcaca cgcgctctgc tcggtgtgga 1020
agcccacgag atcagcatgc tttttctcac cgactacatc aagagtgcca ccggtctcag 1080
taatattgtc tcggacaaga aagacggcgg gcagtatatg cgatgcaaaa caggtgcgtg 1140
cggtgtcctc tcaggtaggg gactcgtttc ttagtggtca ttccaggtat gcagtcgatt 1200
tgccatgcca tgtcaaagga acttgttcca ggctcagtgc acctcaacac ccccgtcgct 1260
ggaattgagc agtcggcgtc cggctgtata gtacgatcgg cctcgggcgc cgtgttccga 1320
agcaaaaagg tggtggtttc gttaccgaca acattgtatc ccaccttgac attttcacca 1380
cctcttcccg ccgagaagca agcattggcg gaaaaatcta tcctcggcta ctatagcaag 1440
atagtcttcg tatgggacaa cccgtggtgg cgcgaacaag gcttctcggg cgtcctccaa 1500
tcgagctgtg accccatctc atttgccaga gataccagca tcgaagtcga tcggcaatgg 1560
tccattacct gtttcatggt cggagacccg ggacggaagt ggtcccaaca gtccaagcag 1620
gtacgacaaa agtctgtctg ggaccaactc cgcgcagcct acgagaacgc cggggcccaa 1680
gtcccagagc cggccaacgt gctcgaaatc gagtggtcga agcagcagta tttccaagga 1740
gctccgagcg ccgtctatgg gctgaacgat ctcatcacac tgggttcggc gctcagaacg 1800
ccgttcaagt gtgttcattt cgttggaacg gagacgtctt tagtttggaa agggtatatg 1860
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gcagcatag 1929

<210> 38
<211> 600
<212> PRT
<213> Exophiala spinifera

<400> 38
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Ala Gly Tyr Ser His Ile Gly Val Gly Pro Asn Glu Ala Arg Tyr Val
20 25 30

Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Leu Gly Val Thr Asp Pro
35 40 45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
50 55 60
Leu Ala Ala Val Gly Ala Ser Ser Asn Asp Val Thr Lys Leu Asn Tyr
65 70 75 80
Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
85 90 95
Leu Lys Ser Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
100 105 110
Pro Val Pro Ala Leu Ala Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
115 120 125
Thr Ala Leu Val Pro Gly His Ser Thr Pro Asp Asn Val Ala Asp Val
130 135 140
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145 150 155 160
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
165 170 175
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
180 185 190
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
195 200 205
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
210 215 220
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
225 230 235 240
Pro Tyr Gly Asp Ser Pro Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
245 250 255
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu Tyr Ser Leu Glu
260 265 270
Asp Pro Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
275 280 285
Ala His Tyr Cys Glu Lys Asp Leu Asn Leu Pro Ala Val Leu Ser Val
290 295 300
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
305 310 315 320
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
325 330 335
Asn Ile Val Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
340 345 350
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
355 360 365
Gly Ser Val His Leu Asn Thr Pro Val Ala Gly Ile Glu Gln Ser Ala
370 375 380
Ser Gly Cys Ile Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
385 390 395 400
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
405 410 415
Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Lys Ser Ile
420 425 430
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Asn Pro Trp Trp
435 440 445
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
450 455 460
Ser Phe Ala Arg Asp Thr Ser Ile Glu Val Asp Arg Gln Trp Ser Ile
465 470 475 480
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
485 490 495
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
500 505 510
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
515 520 525

Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
    530   535   540
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
545   550   555   560
Lys Cys Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
                565   570   575
Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val
            580   585   590
Val Ala Ser Leu Val Pro Ala Ala
        595   600

<210> 39
<211> 1930
<212> DNA
<213> Exophiala spinifera

<220>
<221> intron
<222> (739)...(811)

<221> intron
<222> (1134)...(1187)

<221> misc_feature
<222> (648)...(648)
<223> n = A,T,C or G

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gacgcttcgg gcgtgacaga ccctgcctac gagaaacagg ttgcccaagc attcgccaat 180
ctgcgagctt gccttgctgc agttggagcc acttcaaacg acgtcaccaa gctcaattac 240
tacatcgtcg actacgcccc gagcaaactc accgcaattg gagatgggct gaaggctacc 300
tttgcccttg acaggctccc tccttgcacg ctggtgccag tgtcggcctt gtcttcacct 360
gaatacctct ttgaggttga tgccacggcg ctggtgccgg gacacacgac cccagacaac 420
gttgcggacg tggtagtggt gggcgctggc ttgagcggtt tggagacggc acgcaaagtc 480
caggccgccg gtctgtcctg cctcgttctt gaggcgatgg atcgtgtagg gggaaagact 540
ctgagcgtac aatcgggtcc cggcaggacg actatcaacg acctcggcgc tgcgtggatc 600
aatgacagca accaaagcga agtatccaga ttgtttgaaa gatttcatnt ggagggcgag 660
ctccagagga cgactggaaa ttcaatccat caagcacaag acggtacaac cactacagct 720
ccttatggtg actccttggt aagcacaatc ccactttgtg atgagacctc tgtcgagtgt 780
agaatacagt cactgattcc acttcgtcca gctgagcgag gaggttgcaa gtgcacttgc 840
ggaactcctc cccgtatggt ctcagctgat cgaagagcat agccttcaag acctcaaggc 900
gagccctcag gcgaagcggc tcgacagtgt gagcttcgcg cactactgtg agaaggaact 960
aaacttgcct gctgttctcg gcgtagcaaa ccagatcaca cgcgctctgc tcggtgtgga 1020
agcccacgag atcagcatgc tttttctcac cgactacatc aagagtgcca ccggtctcag 1080
taatattttc tcggacaaga aagacggcgg gcagtatatg cgatgcaaaa caggtgcgtg 1140
tggtgtcgtc tcaggtgggg gactcgtttc tcaagtggtc atttcaggta tgcagtcgat 1200
ttgccatgcc atgtcaaagg aacttgttcc aggctcagtg cacctcaaca cccccgtcgc 1260
tgaaattgag cagtcggcat ccggctgtac agtacgatcg gcctcgggcg ccgtgttccg 1320
aagcaaaaag gtggtggttt cgttaccgac aaccttgtat cccaccttga cattttcacc 1380
acctctcccc gccgagaagc aagcattggc ggaaaattct atcctgggct actatagcaa 1440
gatagtcttc gtatgggaca agccgtggtg gcgcgaacaa ggcttctcgg gcgtcctcca 1500
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gtccattacc tgtttcatgg tcggagaccc gggacggaag tggtcccaac agtccaagca 1620
ggtacgacaa aagtctgtct gggaccaact ccgcgcagcc tacgagaacg ccggggccca 1680
agtcccagag ccggccaacg tgctcgaaat cgagtggtcg aagcagcagt atttccaagg 1740
agctccgagc gccgtctatg ggctgaacga tctcatcaca ctgggttcgg cgctcagaac 1800
gccgttcaag agtgttcatt tcgttggaac ggagacgtct ttagtttgga aagggtatat 1860
ggaaggggcc atacgatcgg gtcaacgagg tgctgcagaa gttgtggcta gcctggtgcc 1920
agcagcatag 1930

<210> 40
<211> 598
<212> PRT
<213> Exophiala spinifera

<220>
<221> VARIANT
<222> (216)...(216)
<223> Xaa = Any Amino Acid

<400> 40
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            20   25   30
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Gly Val Thr Asp Pro
        35   40   45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
    50   55   60
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Val Thr Lys Leu Asn Tyr
65   70   75   80
Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
                85   90   95
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
            100   105   110
Pro Val Ser Ala Leu Ser Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
        115   120   125
Thr Ala Leu Val Pro Gly His Thr Thr Pro Asp Asn Val Ala Asp Val
    130   135   140
Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln
145   150   155   160
Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly
                165   170   175
Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn
            180   185   190
Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Ser
        195   200   205
Arg Leu Phe Glu Arg Phe His Xaa Glu Gly Glu Leu Gln Arg Thr Thr
    210   215   220
Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro
225   230   235   240
Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu
                245   250   255
Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln Asp
            260   265   270
Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe Ala
        275   280   285
His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val Asn
    290   295   300
Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser Met
305   310   315   320
Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn Ile
                325   330   335
Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr Gly
            340   345   350
Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro Gly Ser
        355   360   365
Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser Gly
    370   375   380
Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys Lys Val
385   390   395   400
Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe Ser Pro

405 410 415
Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile Leu Gly
420 425 430
Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg Glu
435 440 445
Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser Phe
450 455 460
Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile Thr Cys
465 470 475 480
Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys Gln
485 490 495
Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr Glu Asn
500 505 510
Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile Glu Trp
515 520 525
Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr Gly Leu
530 535 540
Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe Lys Ser
545 550 555 560
Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr Met
565 570 575
Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val Val Ala
580 585 590
Ser Leu Val Pro Ala Ala
595

<210> 41
<211> 1928
<212> DNA
<213> Rhinocladiella atrovirens

<220>
<221> intron
<222> (739)...(811)

<221> intron
<222> (1134)...(1185)

<400> 41
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cacgtcggcg taggcccaaa cggagggagg tatgcgacaa tagctggaca gattggacaa 120
gacgcttcgg ccgtgacaga ccctgcctac gagaaacagg ttgcccaagc attcgccaac 180
ctgcgagctt gtcttgctgc agttggagcc acttcaaacg acattaccaa gctcaattac 240
tacatcgtcg actacaaccc gagcaaactc accgcaattg gagatgggct gaaggctacc 300
tttgcccttg acaggctccc tccttgcacg ctggtgccag tgccggccct ggcttcacct 360
gaataccccct ttgaggttga tgccacggcg ctggttccag gacactcaac cccagacaat 420
gttgcggacg tggtcgtggt gggcgctggc ttgagcggtt tggagacggc acgcaaagtc 480
caggctgccg ggctgtcctg cctcgttctt gaggcgatgg atcgtgtggg gggaaagact 540
ctgagcgtac aatcgggtcc cggcaggacg gctatcaatg acctcggcgc tgcgtggatc 600
aatgacagca accaaagcga agtattcaaa ttatttgaaa gatttcattt ggagggcgag 660
ctccagagga cgaccggaaa ttcaatccat caagcacaag acggtacaac cactacagct 720
ccttatggtg attccctggt aagcacaatt ccatcttgtg atgagacctc tgtcgtgtgt 780
agaatacagt cgctgactcc acatcgtcca gctgagcgag gaggttgcaa gtgcactcgc 840
ggaactcctt cccgcatggt ctcagctgat cgaagagcat agtcttgaag accccaaggc 900
gagccctcaa gcgaagcagc tcgacagtgt gagcttcgca cactactgtg agaaggatct 960
aagcttgcct gctgttctcg gcgtggcaaa ccagatcaca cgcgctctgc tcggtgtgga 1020
agcccacgag atcagcatgc tttttctcac cgactacatc aagagtgcca ccggtctcag 1080
taatattgtc tcggataaga aagacggtgg gcagtatatg cgatgcaaaa caggtgcgtg 1140
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gccatgccat gtcaaaggaa cttgttccag gctcagtgca cctcaacacc cccgtcgccg 1260
aaattgagca gtcggcatcc ggctgtacag tacgatcggc ctcgggcggc gtgttccgaa 1320
gtaaaaaggt ggtggtttcg ttaccgacaa ccttgtatcc caccttgata ttttcaccac 1380

ctcttcccgc cgagaagcaa gcattggctg aaaaatccat cctgggctac tatagcaaga 1440
tagtcttcgt atgggacaag ccgtggtggc gcgaacaagg cttctcgggc gtcctccaat 1500
cgagctgtga ccccatctca tttgccagag ataccagcat cgaagtcgat cggcaatggt 1560
ccattacctg tttcatggtc ggagacccgg gacggaagtg gtcccaacag tccaagcagg 1620
tacgacagaa gtctgtctgg aaccaactcc gcgcagccta cgagaacgcc ggggcccaag 1680
tcccagagcc ggccaacgtg ctcgagatcg agtggtcgaa gcagcagtat ttccaaggag 1740
cgccgagcgt cgtctatggg ctgaactgtc tcaacacact gggttcggcg ctcagaacgc 1800
cgttcaaggg tgttcatttc gttggaacgg agacgtcttt ggtttggaaa gggtatatgg 1860
aaggggccat acgatcgggt cagcgaggcg ctgcagaagt tgtggctagc ctggtgccag 1920
cagcatag 1928

<210> 42
<211> 598
<212> PRT
<213> Rhinocladiella atrovirens

<400> 42
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Ala Gly Tyr Ser His Val Gly Val Gly Pro Asn Gly Gly Arg Tyr Ala
20 25 30
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Ala Val Thr Asp Pro
35 40 45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
50 55 60
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Ile Thr Lys Leu Asn Tyr
65 70 75 80
Tyr Ile Val Asp Tyr Asn Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
85 90 95
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
100 105 110
Pro Val Pro Ala Leu Ala Ser Pro Glu Tyr Pro Phe Glu Val Asp Ala
115 120 125
Thr Ala Leu Val Pro Gly His Ser Thr Pro Asp Asn Val Ala Asp Val
130 135 140
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145 150 155 160
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
165 170 175
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Ala Ile
180 185 190
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
195 200 205
Phe Lys Leu Phe Glu Arg Leu Glu Gly Glu Leu Gln Arg Thr Thr Gly
210 215 220
Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro Tyr
225 230 235 240
Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu Leu
245 250 255
Leu Pro Ala Trp Ser Gln Leu Ile Glu Glu His Ser Leu Glu Asp Pro
260 265 270
Lys Ala Ser Pro Gln Ala Lys Gln Leu Asp Ser Val Ser Phe Ala His
275 280 285
Tyr Cys Glu Lys Asp Leu Ser Leu Pro Ala Val Leu Gly Val Ala Asn
290 295 300
Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser Met
305 310 315 320
Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn Ile
325 330 335
Val Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr Gly
340 345 350
Met Gln Ser Leu Cys His Ala Met Ser Lys Glu Leu Val Pro Gly Ser

355 360 365
Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser Gly
370 375 380
Cys Thr Val Arg Ser Ala Ser Gly Gly Val Phe Arg Ser Lys Lys Val
385 390 395 400
Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Ile Phe Ser Pro
405 410 415
Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Lys Ser Ile Leu Gly
420 425 430
Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp Arg Glu
435 440 445
Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser Phe
450 455 460
Ala Arg Asp Thr Ser Ile Glu Val Asp Arg Gln Trp Ser Ile Thr Cys
465 470 475 480
Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser Lys Gln
485 490 495
Val Arg Gln Lys Ser Val Trp Asn Gln Leu Arg Ala Ala Tyr Glu Asn
500 505 510
Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile Glu Trp
515 520 525
Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Val Val Tyr Gly Leu
530 535 540
Asn Cys Leu Asn Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe Lys Gly
545 550 555 560
Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly Tyr Met
565 570 575
Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val Val Ala
580 585 590
Ser Leu Val Pro Ala Ala
595

<210> 43
<211> 1928
<212> DNA
<213> Rhinocladiella atrovirens

<220>
<221> intron
<222> (739)...(811)

<221> intron
<222> (1134)...(1186)

<400> 43
atggcacttg caccgagcta catcaatccc ccaaacctcg cctccccagc agggtattcc 60
tacgtcggcg taggcccaaa cggagggagg tatgtgacaa tagctggaca gattggacaa 120
gacgcttcgg ccgtgacaga ccctgcctac gagaaacagg ttgcccaagc attcgccaac 180
ctgcgagctt gtcttgctgc agttggagcc acttcaaacg acattaccaa gctcaattac 240
tacatcgtcg actacaaccc gagcaaactc accgcaattg gagatgggct gaaggctacc 300
tttgcccttg acaggctccc tccttgcacg ctggtgccag tgccggccct ggcttcacct 360
gaatacctct ttgaggttga tgccacggcg ctggttccag gacactcaac cccagacaat 420
gttgcggacg tggtcgtggt gggcgctggc ttgagcggtt tggagacggc acgcaaagtc 480
caggctgccg ggctgtcctg cctcgttctt gaggcgatgg atcgtgtggg gggaaagact 540
ctgagcgtac aatcgggtcc cggcaggacg actatcaatg acctcggcgc tgcgtggatc 600
aatgacagca accaaagcga agtattcaaa ttatttgaaa gatttcattt ggagggcgag 660
ctccagagga cgaccggaaa ttcaatccat caagcacaag acggtacaac cactacagct 720
ccttatggtg attccctggt aagcacaatt ccatcttgtg atgagacctc tgtcgtgtgt 780
agaatacagt cgctgactcc acatcgtcca gctgagcgag gaggttgcaa gtgcactcgc 840
ggaactcctt cccgcatggt ctcagctgat cgaagagcat agtcttgaag accccaaggc 900
gagccctcaa gcgaagcagc tcgacagtgt gagcttcgca cactactgtg agaaggatct 960
aaacttgcct gctgttctcg gcgtggcaaa ccagatcaca cgcgctctgc tcggtgtgga 1020

agcccacgag atcagcatgt tttttctcac cgactacatc aagagtgcca ccggtctcag 1080
taatattgtc tcggataaga aagacggtgg gcagtatatg cgatgcaaaa caggtgcgtg 1140
tggtgttctc tcagtgggag actcgtttct tagtggtcat tccaggtatg cagtcgcttt 1200
gccatgccat gtcaaaggaa cttgttccag gctcagtgca cctcaacacc cccgtcgccg 1260
aaattgagca gtcggcatcc ggctgtacag tacgatcggc ctcgggcggc gtgttccgaa 1320
gtaaaaaggt ggtggtttcg ttaccgacaa ccttgtatcc caccttgata ttttcaccac 1380
ctcttcccgc cgagaagcaa gcattggctg aaaaatccat cctgggctac tatagcaaga 1440
tagtcttcgt atgggacaag ccgtggtggc gcgaacaagg cttctcgggc gtcctccaat 1500
cgagctgtga ccccatctca tttgccagag ataccagcat cgaagtcgat cggcaatggt 1560
ccattacctg tttcatggtc ggagacccgg gacggaagtg gtcccaacag tccaagcagg 1620
tacgacagaa gtctgtctgg aaccaactcc gcgcagccta cgagaacgcc ggggcccaag 1680
tcccagagcc ggccaacgtg ctcgagatcg agtggtcgaa gcagcagtat ttccaaggag 1740
cgccgagcgc cgtctatggg ctgaactgtc tcaacacact gggttcggcg ctcagaacgc 1800
cgttcaaggg tgttcatttc gttggaacgg agacgtcttt ggtttggaaa gggtatatgg 1860
aaggggccat acgatcgggt cagcgaggcg ctgcagaagt tgtggctagc ctggtgccag 1920
cagcatag 1928

<210> 44
<211> 591
<212> PRT
<213> Rhinocladiella atrovirens

<400> 44
Met Ala Leu Ala Pro Ser Tyr Ile Asn Pro Pro Asn Leu Ala Ser Pro
1   5   10   15
Ala Gly Tyr Ser Tyr Val Gly Val Gly Pro Asn Gly Gly Arg Tyr Val
            20   25   30
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Ala Val Thr Asp Pro
        35   40   45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
    50   55   60
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Ile Thr Lys Leu Asn Tyr
65   70   75   80
Tyr Ile Val Asp Tyr Asn Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
                85   90   95
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
            100   105   110
Pro Val Pro Ala Leu Ala Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
        115   120   125
Thr Ala Leu Val Pro Gly His Ser Thr Pro Asp Asn Val Ala Asp Val
    130   135   140
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145   150   155   160
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
                165   170   175
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Gly Arg Thr Thr Ile Asn
            180   185   190
Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Lys
        195   200   205
Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr Gly
    210   215   220
Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro Tyr
225   230   235   240
Gly Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu Leu Leu
                245   250   255
Pro Ala Ser Gln Leu Ile Glu Glu His Ser Leu Glu Asp Pro Lys Ala
            260   265   270
Ser Pro Gln Ala Lys Gln Leu Asp Ser Val Ser Phe Ala His Tyr Cys
        275   280   285
Glu Lys Leu Asn Leu Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg
    290   295   300
Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser Met Phe Phe Leu Thr

305 310 315 320
Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn Ile Val Ser Asp Lys
325 330 335
Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Leu
340 345 350
Cys His Ala Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn
355 360 365
Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg
370 375 380
Ser Ala Ser Gly Gly Val Phe Arg Ser Lys Lys Val Val Leu Pro Thr
385 390 395 400
Leu Tyr Pro Thr Leu Ile Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
405 410 415
Ala Leu Ala Glu Lys Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
420 425 430
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
435 440 445
Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Glu
450 455 460
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
465 470 475 480
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
485 490 495
Asn Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
500 505 510
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
515 520 525
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Cys Leu Asn Thr Leu Gly
530 535 540
Ser Ala Leu Arg Thr Pro Phe Lys Gly Val His Phe Val Gly Thr Glu
545 550 555 560
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
565 570 575
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala
580 585 590

<210> 45
<211> 1928
<212> DNA
<213> Rhinocladiella atrovirens

<220>
<221> intron
<222> (739)...(811)

<221> intron
<222> (1134)...(1185)

<400> 45
atggcacttg caccgagcta catcaatccc ccaaacctcg cctccccagc agggtattcc 60
cacgtcggcg taggcccaaa cggagggagg tatgtgacaa tagctggaca gattggacaa 120
gacgcttcgg ccgtgacaga ccctgcctac gagaaacagg ttgcccaagc attcgccaac 180
ctgcgagctt gtcttgctgc agttggagcc acttcaaacg acattaccaa gctcaattac 240
tacatcgtcg actacaaccc gagcaaactc accgcaattg gagatgggct gaaggctacc 300
tttgcccttg acaggctccc tccttgcacg ctggtgccag tgccggccct ggcttcacct 360
gaatacctct ttgaggttga tgctacggcg ctggttccag gacactcaac cccagacaat 420
gttgcggacg tggtcgtggt gggcgctggc ttgagcggtt tggagacggc acgcaaagtc 480
caggctgccg ggctgtcctg cctcgttctt gaggcgatgg atcgtgtggg gggaaagact 540
ctgagcgtac aatcgggtcc cggcaggacg actatcaatg acctcggcgc tgcgtggatc 600
aatgacagca accaaagcga agtattcaaa ttatttgaaa gatttcattt ggagggcgag 660
ctccagagga cgaccggaaa ttcaatccat caagcacaag acggtacaac cactacagct 720
ccttatggtg attccctggt aggcacaatt ccatcttgtg atgagacctc tgtcgtgtgt 780

agaatacagt cgctgactcc acatcgtcca gctgagcgag gaggttgcaa gtgcactcgc 840
ggaactcctt cccgcatggt ctcagctgat cgaagagcat agtcttgaag accccaaggc 900
gagccctcaa gcgaagcagc tcgacagtgt gagcttcgca cactactgtg agaaggatct 960
aaacttgcct gctgttctcg gcgtggcaaa ccagatcaca cgcgctctgc tcggtgtgga 1020
agcccacgag atcagcatgc tttttctcac cgactacatc aagagtgcca ccggtctcag 1080
taatattgtc tcggataaga aagacggtgg gcagtatatg cgatgcaaaa caggtgcgtg 1140
tggtgttctc tcagtgggag actcgtttct tagtggtcat tccaggtatg cagtcgcttt 1200
gccatgccat gtcaaaggaa cttgttccag gctcagtgca cctcaacacc cccgtcgccg 1260
aaattgagca gtcggcatcc ggctgtacag tacgatcggc ctcgggcggc gtgttccgaa 1320
gtaaaaaggt ggtggtttcg ttaccgacaa ccttgtatcc caccttgata ttttcaccac 1380
ctcttcccgc cgagaagcaa gcattggctg aaaaatccat cctgggctac tatagcaaga 1440
tagtcttcgt atgggacaag ctgtggtggc gcgaacaagg cttctcgggc gtcctccaat 1500
cgagctgtga ccccatctca tttgccagag ataccagcat cgaagtcgat cggcaatggt 1560
ccattacctg tttcatggtc ggagacccgg gacggaagtg gtcccaacag tccaagcagg 1620
tacgacagaa gtctgtctgg aaccaactcc gcgcagccta cgagaacgcc ggggcccaag 1680
tcccagagcc ggccaacgtg ctcgagatcg agtggtcgaa gcagcagtat ttccaaggag 1740
cgccgagcgc cgtctatggg ctgaactgtc tcaacacact gggttcggcg ctcagaacgc 1800
cgttcaaggg tgttcatttc gttggaacgg agacgtcttt ggtttggaaa gggtatatgg 1860
aaggggccat acgatcgggt cagcgaggcg ctgcagaagt tgtgcctagc ctggtgccag 1920
cagcatag 1928

<210> 46
<211> 591
<212> PRT
<213> Rhinocladiella atrovirens

<400> 46
Met Ala Leu Ala Pro Ser Tyr Ile Asn Pro Pro Asn Ala Ser Pro Ala
1   5   10   15
Gly Tyr Ser His Val Gly Val Gly Pro Asn Gly Gly Arg Tyr Val Thr
            20   25   30
Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Ala Val Thr Asp Pro Ala
        35   40   45
Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys Leu
    50   55   60
Ala Ala Val Gly Ala Thr Ser Asn Asp Ile Thr Lys Leu Asn Tyr Tyr
65   70   75   80
Ile Val Asp Tyr Asn Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly Leu
                85   90   95
Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val Pro
            100   105   110
Val Pro Ala Leu Ala Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala Thr
        115   120   125
Ala Leu Val Pro Gly His Ser Thr Pro Asp Asn Val Ala Asp Val Val
    130   135   140
Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val Gln
145   150   155   160
Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val Gly
                165   170   175
Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile Asn
            180   185   190
Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val Phe
        195   200   205
Lys Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr Thr
    210   215   220
Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala Pro
225   230   235   240
Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala Glu
                245   250   255
Leu Leu Pro Ala Trp Ser Gln Leu Ile Glu Glu His Ser Leu Glu Asp
            260   265   270
Pro Lys Ala Ser Pro Gln Ala Lys Gln Leu Asp Ser Val Ser Phe Ala

275 280 285
His Tyr Cys Glu Lys Asp Leu Asn Leu Pro Ala Val Leu Gly Val Ala
290 295 300
Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile Ser
305 310 315 320
Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser Asn
325 330 335
Ile Val Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys Thr
340 345 350
Gly Met Gln Ser Leu Cys His Ala Met Ser Lys Glu Leu Val Pro Gly
355 360 365
Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala Ser
370 375 380
Gly Cys Thr Val Arg Ser Ala Ser Gly Gly Val Phe Arg Ser Lys Lys
385 390 395 400
Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Ile Phe Ser Pro Leu
405 410 415
Pro Ala Glu Lys Gln Ala Leu Ala Glu Lys Ser Ile Gly Tyr Tyr Ser
420 425 430
Lys Ile Val Phe Val Asp Lys Leu Trp Trp Arg Glu Gln Gly Phe Ser
435 440 445
Gly Val Leu Gln Ser Ser Cys Asp Pro Ile Ser Phe Ala Arg Asp Thr
450 455 460
Ser Ile Glu Val Asp Arg Gln Ser Ile Thr Cys Phe Met Val Gly Asp
465 470 475 480
Pro Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val
485 490 495
Trp Asn Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro
500 505 510
Glu Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe
515 520 525
Gln Ala Pro Ser Ala Val Tyr Gly Leu Asn Cys Leu Asn Thr Leu Gly
530 535 540
Ser Ala Leu Arg Thr Pro Phe Lys Gly Val His Phe Val Gly Thr Glu
545 550 555 560
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
565 570 575
Gln Arg Gly Ala Ala Glu Val Val Pro Ser Leu Val Pro Ala Ala
580 585 590

<210> 47
<211> 600
<212> PRT
<213> Exophiala spinifera

<400> 47
Met Ala Leu Ala Pro Ser Tyr Ile Asn Pro Pro Asn Val Ala Ser Pro
1 5 10 15
Ala Gly Tyr Ser His Val Gly Val Gly Pro Asp Gly Gly Arg Tyr Val
20 25 30
Thr Ile Ala Gly Gln Ile Gly Gln Asp Ala Ser Gly Val Thr Asp Pro
35 40 45
Ala Tyr Glu Lys Gln Val Ala Gln Ala Phe Ala Asn Leu Arg Ala Cys
50 55 60
Leu Ala Ala Val Gly Ala Thr Ser Asn Asp Val Thr Lys Leu Asn Tyr
65 70 75 80
Tyr Ile Val Asp Tyr Ala Pro Ser Lys Leu Thr Ala Ile Gly Asp Gly
85 90 95
Leu Lys Ala Thr Phe Ala Leu Asp Arg Leu Pro Pro Cys Thr Leu Val
100 105 110
Pro Val Ser Ala Leu Ser Ser Pro Glu Tyr Leu Phe Glu Val Asp Ala
115 120 125

Thr Ala Leu Val Pro Gly His Thr Thr Pro Asp Asn Val Ala Asp Val
    130   135   140
Val Val Val Gly Ala Gly Leu Ser Gly Leu Glu Thr Ala Arg Lys Val
145   150   155   160
Gln Ala Ala Gly Leu Ser Cys Leu Val Leu Glu Ala Met Asp Arg Val
                165   170   175
Gly Gly Lys Thr Leu Ser Val Gln Ser Gly Pro Gly Arg Thr Thr Ile
            180   185   190
Asn Asp Leu Gly Ala Ala Trp Ile Asn Asp Ser Asn Gln Ser Glu Val
        195   200   205
Ser Arg Leu Phe Glu Arg Phe His Leu Glu Gly Glu Leu Gln Arg Thr
    210   215   220
Thr Gly Asn Ser Ile His Gln Ala Gln Asp Gly Thr Thr Thr Thr Ala
225   230   235   240
Pro Tyr Gly Asp Ser Leu Leu Ser Glu Glu Val Ala Ser Ala Leu Ala
                245   250   255
Glu Leu Leu Pro Val Trp Ser Gln Leu Ile Glu Glu His Ser Leu Gln
            260   265   270
Asp Leu Lys Ala Ser Pro Gln Ala Lys Arg Leu Asp Ser Val Ser Phe
        275   280   285
Ala His Tyr Cys Glu Lys Glu Leu Asn Leu Pro Ala Val Leu Gly Val
    290   295   300
Ala Asn Gln Ile Thr Arg Ala Leu Leu Gly Val Glu Ala His Glu Ile
305   310   315   320
Ser Met Leu Phe Leu Thr Asp Tyr Ile Lys Ser Ala Thr Gly Leu Ser
                325   330   335
Asn Ile Phe Ser Asp Lys Lys Asp Gly Gly Gln Tyr Met Arg Cys Lys
            340   345   350
Thr Gly Met Gln Ser Ile Cys His Ala Met Ser Lys Glu Leu Val Pro
        355   360   365
Gly Ser Val His Leu Asn Thr Pro Val Ala Glu Ile Glu Gln Ser Ala
    370   375   380
Ser Gly Cys Thr Val Arg Ser Ala Ser Gly Ala Val Phe Arg Ser Lys
385   390   395   400
Lys Val Val Val Ser Leu Pro Thr Thr Leu Tyr Pro Thr Leu Thr Phe
                405   410   415
Ser Pro Pro Leu Pro Ala Glu Lys Gln Ala Leu Ala Glu Asn Ser Ile
            420   425   430
Leu Gly Tyr Tyr Ser Lys Ile Val Phe Val Trp Asp Lys Pro Trp Trp
        435   440   445
Arg Glu Gln Gly Phe Ser Gly Val Leu Gln Ser Ser Cys Asp Pro Ile
    450   455   460
Ser Phe Ala Arg Asp Thr Ser Ile Asp Val Asp Arg Gln Trp Ser Ile
465   470   475   480
Thr Cys Phe Met Val Gly Asp Pro Gly Arg Lys Trp Ser Gln Gln Ser
                485   490   495
Lys Gln Val Arg Gln Lys Ser Val Trp Asp Gln Leu Arg Ala Ala Tyr
            500   505   510
Glu Asn Ala Gly Ala Gln Val Pro Glu Pro Ala Asn Val Leu Glu Ile
        515   520   525
Glu Trp Ser Lys Gln Gln Tyr Phe Gln Gly Ala Pro Ser Ala Val Tyr
    530   535   540
Gly Leu Asn Asp Leu Ile Thr Leu Gly Ser Ala Leu Arg Thr Pro Phe
545   550   555   560
Lys Ser Val His Phe Val Gly Thr Glu Thr Ser Leu Val Trp Lys Gly
                565   570   575
Tyr Met Glu Gly Ala Ile Arg Ser Gly Gln Arg Gly Ala Ala Glu Val
            580   585   590
Val Ala Ser Leu Val Pro Ala Ala
        595   600

<210> 48

<211> 1392
<212> DNA
<213> Unknown

<220>
<221> CDS
<222> (1)...(1392)

<223> Cys (-) APAO; removal of cysteine 461

<400> 48
aaa gac aac gtt gcg gac gtg gta gtg gtg ggc gct ggc ttg agc ggt 48
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1 5 10 15

ttg gag acg gca cgc aaa gtc cag gcc gcc ggt ctg tcc tgc ctc gtt 96
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val
20 25 30

ctt gag gcg atg gat cgt gta ggg gga aag act ctg agc gta caa tcg 144
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
35 40 45

ggt ccc ggc agg acg act atc aac gac ctc ggc gct gcg tgg atc aat 192
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
50 55 60

gac agc aac caa agc gaa gta tcc aga ttg ttt gaa aga ttt cat ttg 240
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65 70 75 80

gag ggc gag ctc cag agg acg act gga aat tca atc cat caa gca caa 288
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
85 90 95

gac ggt aca acc act aca gct cct tat ggt gac tcc ttg ctg agc gag 336
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
100 105 110

gag gtt gca agt gca ctt gcg gaa ctc ctc ccc gta tgg tct cag ctg 384
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
115 120 125

atc gaa gag cat agc ctt caa gac ctc aag gcg agc cct cag gcg aag 432
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
130 135 140

cgg ctc gac agt gtg agc ttc gcg cac tac tgt gag aag gaa cta aac 480
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145 150 155 160

ttg cct gct gtt ctc ggc gta gca aac cag atc aca cgc gct ctg ctc 528
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
165 170 175

ggt gtg gaa gcc cac gag atc agc atg ctt ttt ctc acc gac tac atc 576
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
180 185 190

aag agt gcc acc ggt ctc agt aat att ttc tcg gac aag aaa gac ggc 624
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
195 200 205

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ggg cag tat atg cga tgc aaa aca ggt atg cag tcg att tgc cat gcc      672
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala
    210                 215                 220

atg tca aag gaa ctt gtt cca ggc tca gtg cac ctc aac acc ccc gtc      720
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225                 230                 235                 240

gct gaa att gag cag tcg gca tcc ggc tgt aca gta cga tcg gcc tcg      768
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
                245                 250                 255

ggc gcc gtg ttc cga agc aaa aag gtg gtg gtt tcg tta ccg aca acc      816
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
            260                 265                 270

ttg tat ccc acc ttg aca ttt tca cca cct ctt ccc gcc gag aag caa      864
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
        275                 280                 285

gca ttg gcg gaa aat tct atc ctg ggc tac tat agc aag ata gtc ttc      912
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
    290                 295                 300

gta tgg gac aag ccg tgg tgg cgc gaa caa ggc ttc tcg ggc gtc ctc      960
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305                 310                 315                 320

caa tcg agc tcc gac ccc atc tca ttt gcc aga gat acc agc atc gac     1008
Gln Ser Ser Ser Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
                325                 330                 335

gtc gat cga caa tgg tcc att acc tgt ttc atg gtc gga gac ccg gga     1056
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
            340                 345                 350

cgg aag tgg tcc caa cag tcc aag cag gta cga caa aag tct gtc tgg     1104
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
        355                 360                 365

gac caa ctc cgc gca gcc tac gag aac gcc ggg gcc caa gtc cca gag     1152
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
    370                 375                 380

ccg gcc aac gtg ctc gaa atc gag tgg tcg aag cag cag tat ttc caa     1200
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
385                 390                 395                 400

gga gct ccg agc gcc gtc tat ggg ctg aac gat ctc atc aca ctg ggt     1248
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
                405                 410                 415

tcg gcg ctc aga acg ccg ttc aag agt gtt cat ttc gtt gga acg gag     1296
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
            420                 425                 430

acg tct tta gtt tgg aaa ggg tat atg gaa ggg gcc ata cga tcg ggt     1344
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
        435                 440                 445

caa cga ggt gct gca gaa gtt gtg gct agc ctg gtg cca gca gca tag     1392
```

Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala *
    450   455   460

<210> 49
<211> 463
<212> PRT
<213> Unknown

<220>
<223> Cys (-) APAO; removal of cysteine 461

<400> 49
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1   5   10   15
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val
            20   25   30
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
        35   40   45
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
    50   55   60
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65   70   75   80
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
                85   90   95
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
            100   105   110
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
        115   120   125
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
    130   135   140
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145   150   155   160
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
                165   170   175
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
            180   185   190
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
        195   200   205
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Cys His Ala
    210   215   220
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225   230   235   240
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
                245   250   255
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
            260   265   270
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
        275   280   285
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
    290   295   300
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305   310   315   320
Gln Ser Ser Ser Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
                325   330   335
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
            340   345   350
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
        355   360   365
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
    370   375   380
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln

385 390 395 400
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
405 410 415
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
420 425 430
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
435 440 445
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala
450 455 460

<210> 50
<211> 1392
<212> DNA
<213> Unknown

<220>
<221> CDS
<222> (1)...(1392)

<223> Cys (-) APAO; removal of cysteines 359 and 461

<400> 50
aaa gac aac gtt gcg gac gtg gta gtg gtg ggc gct ggc ttg agc ggt 48
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1 5 10 15

ttg gag acg gca cgc aaa gtc cag gcc gcc ggt ctg tcc tgc ctc gtt 96
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val
20 25 30

ctt gag gcg atg gat cgt gta ggg gga aag act ctg agc gta caa tcg 144
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
35 40 45

ggt ccc ggc agg acg act atc aac gac ctc ggc gct gcg tgg atc aat 192
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
50 55 60

gac agc aac caa agc gaa gta tcc aga ttg ttt gaa aga ttt cat ttg 240
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65 70 75 80

gag ggc gag ctc cag agg acg act gga aat tca atc cat caa gca caa 288
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
85 90 95

gac ggt aca acc act aca gct cct tat ggt gac tcc ttg ctg agc gag 336
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
100 105 110

gag gtt gca agt gca ctt gcg gaa ctc ctc ccc gta tgg tct cag ctg 384
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
115 120 125

atc gaa gag cat agc ctt caa gac ctc aag gcg agc cct cag gcg aag 432
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
130 135 140

cgg ctc gac agt gtg agc ttc gcg cac tac tgt gag aag gaa cta aac 480
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145 150 155 160

ttg cct gct gtt ctc ggc gta gca aac cag atc aca cgc gct ctg ctc 528
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
165 170 175

ggt gtg gaa gcc cac gag atc agc atg ctt ttt ctc acc gac tac atc 576
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
180 185 190

aag agt gcc acc ggt ctc agt aat att ttc tcg gac aag aaa gac ggc 624
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
195 200 205

ggg cag tat atg cga tgc aaa aca ggt atg cag tcg att tcg cat gcc 672
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Ser His Ala
210 215 220

atg tca aag gaa ctt gtt cca ggc tca gtg cac ctc aac acc ccc gtc 720
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225 230 235 240

gct gaa att gag cag tcg gca tcc ggc tgt aca gta cga tcg gcc tcg 768
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
245 250 255

ggc gcc gtg ttc cga agc aaa aag gtg gtg gtt tcg tta ccg aca acc 816
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
260 265 270

ttg tat ccc acc ttg aca ttt tca cca cct ctt ccc gcc gag aag caa 864
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
275 280 285

gca ttg gcg gaa aat tct atc ctg ggc tac tat agc aag ata gtc ttc 912
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
290 295 300

gta tgg gac aag ccg tgg tgg cgc gaa caa ggc ttc tcg ggc gtc ctc 960
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305 310 315 320

caa tcg agc tcc gac ccc atc tca ttt gcc aga gat acc agc atc gac 1008
Gln Ser Ser Ser Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
325 330 335

gtc gat cga caa tgg tcc att acc tgt ttc atg gtc gga gac ccg gga 1056
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
340 345 350

cgg aag tgg tcc caa cag tcc aag cag gta cga caa aag tct gtc tgg 1104
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
355 360 365

gac caa ctc cgc gca gcc tac gag aac gcc ggg gcc caa gtc cca gag 1152
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
370 375 380

ccg gcc aac gtg ctc gaa atc gag tgg tcg aag cag cag tat ttc caa 1200
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
385 390 395 400

gga gct ccg agc gcc gtc tat ggg ctg aac gat ctc atc aca ctg ggt 1248
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly

405 410 415

tcg gcg ctc aga acg ccg ttc aag agt gtt cat ttc gtt gga acg gag 1296
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
420 425 430

acg tct tta gtt tgg aaa ggg tat atg gaa ggg gcc ata cga tcg ggt 1344
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
435 440 445

caa cga ggt gct gca gaa gtt gtg gct agc ctg gtg cca gca gca tag 1392
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala *
450 455 460

<210> 51
<211> 463
<212> PRT
<213> Unknown

<220>
<223> Cys (-) APAO; removal of cysteines 359 and 461

<400> 51
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1 5 10 15
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Cys Leu Val
20 25 30
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
35 40 45
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
50 55 60
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65 70 75 80
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
85 90 95
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
100 105 110
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
115 120 125
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
130 135 140
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145 150 155 160
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
165 170 175
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
180 185 190
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
195 200 205
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Ser His Ala
210 215 220
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225 230 235 240
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
245 250 255
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
260 265 270
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
275 280 285
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
290 295 300

Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305 310 315 320
Gln Ser Ser Ser Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
325 330 335
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
340 345 350
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
355 360 365
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
370 375 380
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
385 390 395 400
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
405 410 415
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
420 425 430
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
435 440 445
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala
450 455 460

<210> 52
<211> 1392
<212> DNA
<213> Unknown

<220>
<221> CDS
<222> (1)...(1392)

<223> Cys (-) APAO; removal of cysteines 169, 359, and
461

<400> 52
aaa gac aac gtt gcg gac gtg gta gtg gtg ggc gct ggc ttg agc ggt 48
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1 5 10 15

ttg gag acg gca cgc aaa gtc cag gcc gcc ggt ctg agc tcc ctc gtt 96
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Ser Leu Val
20 25 30

ctt gag gcg atg gat cgt gta ggg gga aag act ctg agc gta caa tcg 144
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
35 40 45

ggt ccc ggc agg acg act atc aac gac ctc ggc gct gcg tgg atc aat 192
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
50 55 60

gac agc aac caa agc gaa gta tcc aga ttg ttt gaa aga ttt cat ttg 240
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65 70 75 80

gag ggc gag ctc cag agg acg act gga aat tca atc cat caa gca caa 288
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
85 90 95

gac ggt aca acc act aca gct cct tat ggt gac tcc ttg ctg agc gag 336
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
100 105 110

gag gtt gca agt gca ctt gcg gaa ctc ctc ccc gta tgg tct cag ctg 384
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
115 120 125

atc gaa gag cat agc ctt caa gac ctc aag gcg agc cct cag gcg aag 432
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
130 135 140

cgg ctc gac agt gtg agc ttc gcg cac tac tgt gag aag gaa cta aac 480
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145 150 155 160

ttg cct gct gtt ctc ggc gta gca aac cag atc aca cgc gct ctg ctc 528
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
165 170 175

ggt gtg gaa gcc cac gag atc agc atg ctt ttt ctc acc gac tac atc 576
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
180 185 190

aag agt gcc acc ggt ctc agt aat att ttc tcg gac aag aaa gac ggc 624
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly
195 200 205

ggg cag tat atg cga tgc aaa aca ggt atg cag tcg att tcg cat gcc 672
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Ser His Ala
210 215 220

atg tca aag gaa ctt gtt cca ggc tca gtg cac ctc aac acc ccc gtc 720
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225 230 235 240

gct gaa att gag cag tcg gca tcc ggc tgt aca gta cga tcg gcc tcg 768
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
245 250 255

ggc gcc gtg ttc cga agc aaa aag gtg gtg gtt tcg tta ccg aca acc 816
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
260 265 270

ttg tat ccc acc ttg aca ttt tca cca cct ctt ccc gcc gag aag caa 864
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
275 280 285

gca ttg gcg gaa aat tct atc ctg ggc tac tat agc aag ata gtc ttc 912
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
290 295 300

gta tgg gac aag ccg tgg tgg cgc gaa caa ggc ttc tcg ggc gtc ctc 960
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305 310 315 320

caa tcg agc tcc gac ccc atc tca ttt gcc aga gat acc agc atc gac 1008
Gln Ser Ser Ser Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
325 330 335

gtc gat cga caa tgg tcc att acc tgt ttc atg gtc gga gac ccg gga 1056
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
340 345 350

cgg aag tgg tcc caa cag tcc aag cag gta cga caa aag tct gtc tgg 1104
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp

355 360 365

gac caa ctc cgc gca gcc tac gag aac gcc ggg gcc caa gtc cca gag 1152
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
370 375 380

ccg gcc aac gtg ctc gaa atc gag tgg tcg aag cag cag tat ttc caa 1200
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
385 390 395 400

gga gct ccg agc gcc gtc tat ggg ctg aac gat ctc atc aca ctg ggt 1248
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
405 410 415

tcg gcg ctc aga acg ccg ttc aag agt gtt cat ttc gtt gga acg gag 1296
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
420 425 430

acg tct tta gtt tgg aaa ggg tat atg gaa ggg gcc ata cga tcg ggt 1344
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
435 440 445

caa cga ggt gct gca gaa gtt gtg gct agc ctg gtg cca gca gca tag 1392
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala *
450 455 460

<210> 53
<211> 463
<212> PRT
<213> Unknown

<220>
<223> Cys (-) APAO; removal of cysteines 169, 359, and
461

<400> 53
Lys Asp Asn Val Ala Asp Val Val Val Val Gly Ala Gly Leu Ser Gly
1 5 10 15
Leu Glu Thr Ala Arg Lys Val Gln Ala Ala Gly Leu Ser Ser Leu Val
20 25 30
Leu Glu Ala Met Asp Arg Val Gly Gly Lys Thr Leu Ser Val Gln Ser
35 40 45
Gly Pro Gly Arg Thr Thr Ile Asn Asp Leu Gly Ala Ala Trp Ile Asn
50 55 60
Asp Ser Asn Gln Ser Glu Val Ser Arg Leu Phe Glu Arg Phe His Leu
65 70 75 80
Glu Gly Glu Leu Gln Arg Thr Thr Gly Asn Ser Ile His Gln Ala Gln
85 90 95
Asp Gly Thr Thr Thr Thr Ala Pro Tyr Gly Asp Ser Leu Leu Ser Glu
100 105 110
Glu Val Ala Ser Ala Leu Ala Glu Leu Leu Pro Val Trp Ser Gln Leu
115 120 125
Ile Glu Glu His Ser Leu Gln Asp Leu Lys Ala Ser Pro Gln Ala Lys
130 135 140
Arg Leu Asp Ser Val Ser Phe Ala His Tyr Cys Glu Lys Glu Leu Asn
145 150 155 160
Leu Pro Ala Val Leu Gly Val Ala Asn Gln Ile Thr Arg Ala Leu Leu
165 170 175
Gly Val Glu Ala His Glu Ile Ser Met Leu Phe Leu Thr Asp Tyr Ile
180 185 190
Lys Ser Ala Thr Gly Leu Ser Asn Ile Phe Ser Asp Lys Lys Asp Gly

195 200 205
Gly Gln Tyr Met Arg Cys Lys Thr Gly Met Gln Ser Ile Ser His Ala
210 215 220
Met Ser Lys Glu Leu Val Pro Gly Ser Val His Leu Asn Thr Pro Val
225 230 235 240
Ala Glu Ile Glu Gln Ser Ala Ser Gly Cys Thr Val Arg Ser Ala Ser
245 250 255
Gly Ala Val Phe Arg Ser Lys Lys Val Val Val Ser Leu Pro Thr Thr
260 265 270
Leu Tyr Pro Thr Leu Thr Phe Ser Pro Pro Leu Pro Ala Glu Lys Gln
275 280 285
Ala Leu Ala Glu Asn Ser Ile Leu Gly Tyr Tyr Ser Lys Ile Val Phe
290 295 300
Val Trp Asp Lys Pro Trp Trp Arg Glu Gln Gly Phe Ser Gly Val Leu
305 310 315 320
Gln Ser Ser Ser Asp Pro Ile Ser Phe Ala Arg Asp Thr Ser Ile Asp
325 330 335
Val Asp Arg Gln Trp Ser Ile Thr Cys Phe Met Val Gly Asp Pro Gly
340 345 350
Arg Lys Trp Ser Gln Gln Ser Lys Gln Val Arg Gln Lys Ser Val Trp
355 360 365
Asp Gln Leu Arg Ala Ala Tyr Glu Asn Ala Gly Ala Gln Val Pro Glu
370 375 380
Pro Ala Asn Val Leu Glu Ile Glu Trp Ser Lys Gln Gln Tyr Phe Gln
385 390 395 400
Gly Ala Pro Ser Ala Val Tyr Gly Leu Asn Asp Leu Ile Thr Leu Gly
405 410 415
Ser Ala Leu Arg Thr Pro Phe Lys Ser Val His Phe Val Gly Thr Glu
420 425 430
Thr Ser Leu Val Trp Lys Gly Tyr Met Glu Gly Ala Ile Arg Ser Gly
435 440 445
Gln Arg Gly Ala Ala Glu Val Val Ala Ser Leu Val Pro Ala Ala
450 455 460